PB84-162866

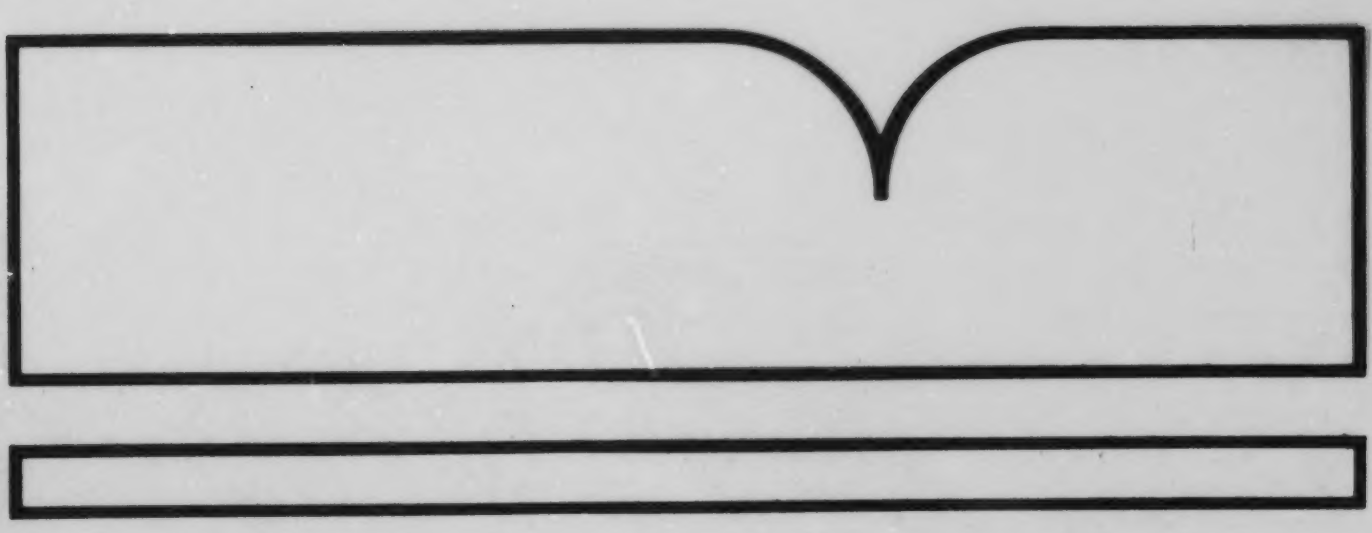
Microbial Transformation Kinetics of Xenobiotics in Aquatic Environment

Battelle Pacific Northwest Labs., Richland, WA

Prepared for

Environmental Research Lab., Athens, GA

Mar 84



U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-162866

EPA-600/3-84-043
March 1984

MICROBIAL TRANSFORMATION KINETICS OF XENOBIOTICS IN AQUATIC ENVIRONMENT

by

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Cooperative Agreement No. CR810436

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ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
ATHENS, GEORGIA 30613

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/3-84-043	2.
3. RECIPIENT'S ACCESSION NO. PB84 162866	
4. TITLE AND SUBTITLE Microbial Transformation Kinetics of Xenobiotics in Aquatic Environment	5. REPORT DATE March 1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) John E. Rogers, Shu-mei W. Li, and Lawrence J. Felice	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Battelle Pacific Northwest Laboratories Richland WA 99352	10. PROGRAM ELEMENT NO. CCBE1A
	11. CONTRACT/GRANT NO. CR810436
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency--Athens GA Office of Research and Development Environmental Research Laboratory Athens GA 30613	13. TYPE OF REPORT AND PERIOD COVERED Final, 10/82-12/83
	14. SPONSORING AGENCY CODE EPA/600/01
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

The microbiological transformation rates of four organic compounds, the butoxyethylester of 2,4-dichlorophenoxyacetic acid (2,4-DBE), p-cresol, α-naphthol and quinoline, added to natural water samples were examined in laboratory experiments. Graphical analysis of the data with first-order log plots indicated that transformation of these compounds occurred in two phases. The initial phase consisted of a lag period during which no decrease in compound concentration could be detected. Three of the compounds--p-cresol, α-naphthol and quinoline-- were only transformed following a lag phase. The transformation of 2,4-DBE occurred immediately upon addition of the compound to sample waters. The lag period was followed by a transformation phase where the detectable decrease in compound concentration could be described by a pseudo first-order rate equation and for which psuedo first-order constants could be determined. The variability in first-order constants for the different compounds ranged from a low of 13.6-fold for 2,4-DBE to a high of 185-fold for quinoline. Much of the variability could be accounted for in the range of average bacterial populations, measured during the transformation phase, that were used to calculate second-order rate constants and from the observation that second-order rate constants could be clustered into groups that were statistically different. The variability of second-order constants within these groups ranged from 1.18 to 36.14-fold, whereas the first-order constants ranged from 1.24 to 184.71-fold.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 104
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

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FOREWORD

Environmental protection efforts are increasingly directed towards prevention of adverse health and ecological effects associated with specific compounds of natural or human origin. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Environmental Processes Branch studies the microbiological, chemical, and physico-chemical processes that control the transport transformation, and impact of pollutants in soil and water.

In recent years, attempts have been made to quantitate microbiological degradation and transformation rates for numerous organics in the aquatic environment. A number of mathematical models for estimating persistence of organics have resulted from these studies. To expand the application of these models, second-order rate equations have been used to provide the required transformation rate data but better information has been needed regarding the reliability of these equations. In the research described here, the microbiological transformation of a series of organic substrates was examined to determine whether their rate of degradation is reasonably described by second-order rate equations developed in laboratory studies.

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ABSTRACT

The microbiological transformation rates of four organic compounds, the butoxyethylester of 2,4-dichlorophenoxyacetic acid (2,4-DBE), p-cresol, α-naphthol and quinoline, added to natural water samples were examined in laboratory experiments. Graphical analysis of the data with first-order log plots indicated that transformation of these compounds occurred in two phases. The initial phase consisted of a lag period during which no decrease in compound concentration could be detected. Three of the compounds p-cresol, α-naphthol and quinoline were only transformed following a lag phase. The transformation of 2,4-DBE occurred immediately upon addition of the compound to sample waters. The lag period was followed by a transformation phase where the detectable decrease in compound concentration could be described by a pseudo first-order rate equation and for which pseudo first-order rate constants could be determined. The variability in first-order constants for the different compounds ranged from a low of 13.6-fold for 2,4-DBE to a high of 185-fold for quinoline. Much of the variability could be accounted for in the range in average bacterial populations, measured during the transformation phase, that were used to calculate second-order rate constants and from the observation that second-order rate constants could be clustered into groups that were statistically different. The variability of second-order constants within these groups ranged from 1.18 to 36.14-fold, whereas the first-order constants ranged from 1.24 to 184.71-fold. Within all but two groups the range in first-order rate constants was greater than the range in second-order constants.

This report was submitted in fulfillment of Cooperative Agreement No. CR810436 by Battelle, Pacific Northwest Laboratories, under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from October 1982 to December 1983, and work was completed as of December 1983.

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INTRODUCTION

The development of viable research and predictive models for the degradation of organic compounds in the environment requires the interaction of the following two research areas.

- The development of environmentally applicable mathematical representations of the microbiological processes involved in the transformation and ultimate degradation of organic compounds.

- The design and development of procedures to produce laboratory data compatible with the mathematical representations.

An important first step in this direction has been the use of the second-order rate equation to describe the microbiological transformation of organics in fresh and marine waters. The equation is limited and is most applicable when the organic substrate concentration is less than saturating (assuming that microorganisms can be considered catalysts) and the substrate does not support growth of the microbial population. However, considering these two constraints, the utility of this equation has been demonstrated over a large number of waters for several compounds that include a number of phenol derivatives (Paris et al., 1983), malathion, the butoxyethylester of 2,4-dichlorophenoxyacetic acid and chloropropham (Paris et al., 1981). The coefficient of variation for second-order rate constants for each of the compounds was less than 65% for all sample waters. This reproducibility has offered significant encouragement for the prediction of the environmental fate in natural waters of contaminating organics that meet the constraints listed above.

The ultimate utility of second-order rate equations and second-order rate constants in predicting the transformation rates of organics under differing environmental conditions will depend on: (1) reproducibility of second-order rate constants experimentally determined in different laboratories; and (2) range of compound classes for which second-order rate equations will describe their transformation in aquatic environments. We therefore initiated a research program to examine the microbiological transformation of a series of organic substrates and to determine if, in our laboratory, the rate of degradation of these compounds was reasonably described by the second-order rate equation. We chose to examine the transformation of α-naphthol, quinoline, p-cresol, malathion and the butoxyethylester of 2,4-dichlorophenoxyacetic acid. The latter three compounds have been studied

extensively at the EPA, Athens, Environmental Research Laboratory and were included in the study for interlaboratory comparison. The remaining two compounds were chosen to represent two new compound classes, nitrogen heterocycles (quinoline) and multiple ring phenolics (α-naphthol).

MATERIALS AND METHODS

Water Collection

Four to eight liters of water were collected periodically from seven different sites within 100 km of the Battelle, Pacific Northwest Laboratories. Both temperature and oxygen concentration were measured with a Yellow Springs Instruments oxygen meter (Model 57) at the time of collection. The samples were transferred back to the laboratory in sterile glass jugs, which were kept in ice chests. Upon arriving at the laboratory they were immediately dispensed into incubation flasks.

At the laboratory, pH was measured with an Orion Model 611 meter with a 810200 (Ross) combination probe; total organic carbon was quantified using a Dohrmann DC-80 carbon analyzer; conductivity was measured with a Beckman RC-20 meter; total P was determined by inductively coupled plasma emission spectrometry. The anions, sulfate and nitrate, were quantified with a Dionex Model 16 ion chromatograph with conductivity detection.

Chemicals

Malathion (99.8% purity) was a gift from the American Cyanamide Co., Princeton, New Jersey. The butoxyethylester of 2,4-dichlorophenoxyacetic acid (99.78% purity) was a gift from Dow Chemical Co. Quinoline (99% purity), p-cresol (99% purity) and α-naphthol (99% purity) were obtained from the Aldrich Chemical Co. All chemicals were used without further purification, except α-naphthol, which was purified by sublimation. Nutrient broth and tryptone glucose extract agar were obtained from Difco Laboratories.

All stock solutions of test compounds, with the exception of 2,4-DBE, were prepared by dissolving a measured amount of test compound into distilled water and subsequently filter-sterilizing the sample through a 0.22 μ filter-membrane. Stock solution concentrations were adjusted so addition of the test compound would not change the volume of the water sample more than 0.25%. Because of extremely low solubility in water, 2,4-DBE was prepared fresh for each experiment using a filter (0.22 μ membrane) sterilized aliquot of the water under study. This procedure avoided a 10% dilution of nutrients in the preparation of water samples for transformation experiments.

Design of Transformation Experiments

A measured volume of 400 mL of a water sample was added to each of three 1 L incubation flasks. To two of the flasks, the test compound was added to give a final concentration which ranged from 1.0 to 0.01 mg of compound per liter. If more than one concentration was investigated, two additional flasks were included for each additional concentration. The remaining unamended flask served as a control to determine changes in bacterial populations not due to the test compound. Control flasks containing 400 mL of filter-sterilized water amended with the test compound at the appropriate concentration were included to estimate abiotic processes. Flasks were incubated in a temperature controlled incubator shaker; the temperature was maintained at 12 or 20°C depending on what the temperature of the water was closest to at the time of sampling. Sampling schedules for chemical analysis were arranged so 10 to 12 samples were generally collected through at least two half-lives of the parent compound. Bacterial levels were determined at alternate sampling periods.

Sequential Amendment Experiments

In several instances, long and variable adaptation periods were observed before transformation of the test compound was observed. The variability in the adaption period required that a far greater number of samples than generally required be taken for analysis of residual organic substrate to bracket a time interval within which transformation might be expected to occur. An experimental protocol requiring repeated amendment of the organic substrate to the reaction vessel has been investigated to circumvent these difficulties. Each new amendment was added after the concentration of the previous amendment had fallen to a less than detectable value. This procedure reduced the total number of samples required and defined, with greater efficiency, the time period when transformation of the test substrate was optimal.

Sequential amendment experiments, which closely followed the protocol established for transformation studies, contained several procedural modifications. Water samples were monitored daily for residual levels of the test compound. Water samples found to contain less than detectable concentrations of the test compound were reamended to the original concentration and subsequently monitored with more rigorous sampling scheme. Both the residual concentration of the test compound and the bacterial concentration were monitored during this period.

Enhanced Microbial Populations

For the more recalcitrant compounds, transformation studies were conducted with enhanced microbial populations. River sediment was combined with river water (5 to 20% w/v) and blended (high speed) 4 X 15 sec with 15 sec intermissions. The mixture was stored in a refrigeration unit (5°C) for 24 hours before the upper 66% of the mixture was decanted. The supernatant solution was mixed well and subdivided into individual incubation flasks as in river water experiments.

Enhanced populations were prepared by amending river water samples with 10 ppm nutrient broth and incubating the amended culture for 24 hours before the addition of the test compound.

Bacterial Enumeration

Bacterial concentrations were determined by pour plate techniques (American Public Health Association, 1971) using tryptone glucose extract agar. Plates were incubated at 22°C and were enumerated after a minimum incubation period of 48 hours. Bacterial concentrations represent the average of three plates and are expressed as colony forming units (cfu) per liter.

Quantitative Organic Analysis

Water samples for analysis of p-Cresol, α-naphthol, and quinoline were centrifuged at 12000 X g for 10 min before 2 mL of the supernatant solution was subsampled for analysis by high performance liquid chromatography. To maintain the sterility of the 2 mL subsamples during analysis, 10 µL chloroform was added to each subsample.

Direct liquid chromatographic analysis of water samples was used in the quantitation of residual concentrations. Subsamples were chromatographed on a C_{18} column (Water's) with a 40% acetonitrile, 60% 0.1 M citrate buffer (1:1, citric acid, Na_3citrate) mobile phase. Samples were injected automatically using a Water's WISP Model 710B injector. p-Cresol and α-naphthol were detected with an amperometric detector (Bioanalytical Systems, Inc., Model LC4A) operating in the oxidative mode using a glassy carbon electrode (+0.9 V vs. Ag/AgCl). Quinoline was detected with a UV monitor (Water's Model 450) set at 230 nm. In both cases detector responses were monitored with a Hewlett-Packard Model 3390A integrator and quantified using peak areas.

Malathion and 2,4-DBE were first extracted from sample solutions by iso-octane and residual concentrations of malathion and 2,4-DBE in

iso-octane extracts were analyzed by capillary gas chromatography with electron capture detection (^{63}Ni) on a Hewlett-Packard 5840A gas chromatograph. Samples were automatically injected (HP model 7672A autosampler) onto a 60 meter SE54 column (J&W Scientific, Inc.) in the splitless mode. Helium was the carrier gas (1 cc/min) and 10% methane in argon was the make-up gas (20 cc/min). The injector temperature was 225°C and the detector temperature was 325°C. The oven temperature was programmed in the following manner: initial temp. = 70°C (4 min), final temp. = 250°C (10 min); rate = 10°C/min for the first 10 min and rate = 4°C/min for the remainder of the program. Peak area responses were electronically integrated by the gas chromatograph. Because of high background levels of di-n-butylphthalate, all glassware used in malathion experiments was acid washed with 2 N HCl. At a ratio of 1:1 iso-octane to sample, extraction efficiency was 96 to 99%. No sample cleanup was necessary.

All compounds investigated (p-cresol, α-naphthol, quinoline, 2,4-DBE, and malathion) were quantitated against standard solutions of known concentration.

Degradation Kinetics

Pseudo first-order rate constants (k) for 2,4-DBE were obtained from the linear least squares analysis of a log (initial/residual concentration) vs. time first-order plot. Because a lag period was observed before the transformation of p-cresol, α-naphthol and quinoline was detected, pseudo first-order rate constants for these compounds were determined form the linear portion of the transformation phase. When linearity was not observed the first-order constant was determined from the last three data points with the knowledge that it would be a conservative estimate. Second-order rate constants (k) were obtained by dividing k by the bacterial concentration. The bacterial concentration was the average of bacterial counts obtained over the time interval used for the determination of the first-order rate constants.

RESULTS

Characteristics of Collected Waters

A total of seven sites divided among four rivers, the Columbia, Snake, Yakima and Walla Walla, were selected for water collection. Over a period of nine months, 11-8-1982 to 8-7-1983, 48 samples were collected. Table 1 shows the characteristics of the waters.

Butoxyethylester of 2,4-Dichlorophenoxyacetic Acid

A total of six waters were examined in duplicate for the transformation of 2,4-DBE initially present at 100 ppb. The data collected in these experiments can be found in Appendix A. In all cases transformation was observed immediately after the addition of 2,4-DBE, i.e. adaptation of the bacterial population to 2,4-DBE was not required before transformation could occur.

Table 1. Characteristics of collected waters

Characteristics	Range
Temperature (°C)	3 to 24
pH	6.5 to 8.9
Conductivity (μmho)	141 to 613
Bacterial population (cfu/L)	5.7×10^4 to 1.8×10^7
Dissolved oxygen (ppm)	6.5 to 14.8
Total organic carbon (ppm)	1.1 to 18.8
Total P (ppm)	0.003 to 7.7
NO_3^- (ppm)	0.29 to 6.69
SO_4^{2-} (ppm)	7.07 to 50.9

Pseudo first-order constants derived from first-order log plots (Appendix A) ranged from a low of 0.030 per hr to a high of 0.407 per hr. Much of the variability in the first-order constants was accounted for in the range of bacterial populations (1.19×10^6 to 2.81×10^7 cfu/L) observed for the different waters. Second-order rate constants (Table 2) determined from the ratio of pseudo first-order rate constant to the average bacterial population appeared to fall into two groups. The first comprises nine water samples for which second-order constants ranged from 8.54×10^{-9} to 2.55×10^{-8} L organism^{-1} hr^{-1}. The second contains the remaining constants derived from the Columbia River (2-14-82) replicate 2 and the Snake River (3-11-83) replicates 1 and 2, which ranged in value from 7.48×10^{-8} to 2.97×10^{-7} L organism^{-1} hr^{-1}. Each of the constants in the second group was distinct from the first group when examined individually by the Q test at the 0.99 confidence level. The mean second-order rate constant for Group I [$K = (1.61 \pm 0.64) \times 10^{-8}$ L organism^{-1} hr^{-1}] was approximately 10 times less than the mean constant for Group II [$K = (2.07 \pm 1.17) \times 10^{-7}$ L organism^{-1} hr^{-1}]. It is significant to note that the range in second-order constants of Group I was reduced in comparison to the variability in the pseudo first-order constants (Table 3), which ranged from 0.030 to 0.253 per hr. A similar reduction was not observed for Group II constants, however, this group only contained 3 values.

p-Cresol

Transformation experiments were completed for six waters. The data collected in these studies can be found in Appendix B and Tables 6 and 7. In most experiments a significant lag period was observed before a detectable decrease in the p-cresol could be measured. The length of the lag period varied with the p-cresol concentration and the water sample. For a given water the lag period observed at 1.0 ppm p-cresol was 20 to 30 hr longer than at 0.1 ppm. With different waters the lag ranged from 30 to 66 hr at 1.0 ppm p-cresol and 2 to 47 hr at 0.1 ppm.

Pseudo first-order rate constants were generally determined form the portion of the first order plot where a linear decrease in p-cresol concentration was observed. In some experiments greater than 50% of the amended p-cresol was transformed before linearity in the plot was observed and in a few cases a linear portion of the plot could not be readily identified. In these cases, the rate constant was determined from a linear least squares fit of the last three data points, with the understanding that this procedure led to a conservative estimate of the first-order rate constant.

Table 2. Summary of second-order rate constants for butoxyethyl ester of 2,4-dichlorophenoxyacetic acid

Water Sample	Initial Concentration (ppb)	Replicate	K (liters organism^{-1} hr^{-1})
Group I			
331 Bldg. Fish Pond (1-31-83)	100	1	2.34×10^{-8}
	100	2	1.55×10^{-8}
331 Bldg. Fish Pond (2-8-83)	100	1	2.55×10^{-8}
	100	2	8.54×10^{-9}
Columbia River Richland "Wye" Marina (2-14-83)	100	1	2.21×10^{-8}
Columbia River Richland "Wye" Marina (2-28-83)	100	1	1.26×10^{-8}
	100	2	1.07×10^{-8}
Yakima River - Horn Rapids Park Boat Ramp (3-1-83)	100	1	1.75×10^{-8}
	100	2	8.89×10^{-9}
		MEAN	$(1.61 \pm 0.64) \times 10^{-8}$
Group II			
Columbia River Richland "Wye" Marina (2-14-83)	100	2	7.48×10^{-8}
Snake River - Hood Park Fish Pond (3-11-83)	100	1	2.50×10^{-7}
	100	2	2.97×10^{-7}
		MEAN	$(2.07 \pm 1.17) \times 10^{-7}$

Table 3. Comparison of pseudo first-order and second-order rate constants for butoxyethyl ester of 2,4-dichlorophenoxyacetic acid

	No. of Constants Compared	Range of Constants	
		Pseudo First-Order (hr^{-1})	Second-Order (liters $organism^{-1}$ hr^{-1})
Group I	(9)	0.030 to 0.253	8.54×10^{-9} to 2.55×10^{-8}
		(8.43)[a]	(2.99)
Group II	(3)	0.240 to 0.407	7.48×10^{-8} to 2.97×10^{-7}
		(1.70)	(3.97)

[a]ratio of highest and lowest values

Table 4. Summary of second-order rate constants for p-cresol

Water Sample	Initial Concentration (ppb)	Replicate	K (liters organism^{-1} hr^{-1})
Group I			
331 Bldg. Fish Pond (12-3-82)	1,000	1	4.01×10^{-9}
	1,000	2	2.22×10^{-9}
	100	1	2.80×10^{-9}
	100	2	2.37×10^{-9}
331 Bldg. Fish Pond (1-14-83)	1,000	1	1.06×10^{-9}
	1,000	2	2.17×10^{-9}
	100	1	6.86×10^{-9}
Yakima River - Horn Rapids Park Boat Ramp (2-7-83)	1,000	1	8.73×10^{-9}
	1,000	2	2.20×10^{-9}
	100	1	3.34×10^{-9}
	100	2	4.25×10^{-9}
Columbia River Richland "Wye" Marina (2-14-83)	1,000	2	5.76×10^{-9}
Yakima River - Horn Rapids Park Boat Ramp (4-22-83)	1,000	1	6.96×10^{-9}
	1,000	2	1.15×10^{-8}
		MEAN	$(4.59 \pm 3.00) \times 10^{-9}$
Group II			
331 Bldg. Fish Pond (1-14-83)	100	2	2.78×10^{-8}
Columbia River Richland "Wye" Marina (2-14-83)	1,000	1	1.87×10^{-8}
Walla Walla River (3-28-83)	1,000	1	2.32×10^{-8}
	1,000	2	2.59×10^{-8}
Yakima River - Horn Rapids Park Boat Ramp (4-22-83)	100	1	5.42×10^{-8}
	100	2	5.97×10^{-8}
		MEAN	$(3.49 \pm 1.74) \times 10^{-8}$

Table 5. Comparison of pseudo first-order and second-order rate constants for p-cresol

	No. of Constants Compared	Range of Constants	
		Pseudo First-Order (hr^{-1})	Second-Order (liters organism^{-1} hr^{-1})
Group I	(14)	0.041 to 0.790	1.06×10^{-9} to 1.15×10^{-8}
		(19.27)[a]	(10.85)
Group II	(6)	0.082 to 0.340	1.87×10^{-8} to 5.97×10^{-8}
		(4.15)	(3.19)

[a]ratio of highest and lowest values

Table 6. Sequential amendment study, 1000 ppb p-cresol, Yakima River - Horn Rapids Park boat ramp (4-22-83)

Incubation Time (hr)		Replicate 1 Init./Res.	Log		Replicate 2 Init./Res.	Log
48		1.32	0.12*[a]		1.27	0.10*
49		1.37	0.14*		0.93	-0.03
50		0.81	-0.09		0.74	-0.13
51		1.65	0.22*		sl	sl[b]
52		1.43	0.16*		1.41	0.15*
53		1.66	0.22*		1.66	0.22*
54		1.76	0.25*		1.68	0.23*
55		1.68	0.23*		1.68	0.23*
56		1.96	0.29*		2.04	0.31*
58		2.38	0.38*		2.20	0.34*
60		2.94	0.47*		3.03	0.48*
62		3.45	0.54*		4.40	0.64*
72	2nd Amend. →	0.98	-0.01	2nd Amend. →	1.15	0.06
73		1.08	0.03		1.22	0.09
74		1.19	0.08		0.90	-0.05
75		1.48	0.17		1.62	0.21
76		1.59	0.20		2.14	0.33
77		1.72	0.24*		2.33	0.37*
78		2.76	0.44*		5.22	0.72*
79		3.61	0.56*		7.20	0.86*
96	3rd Amend. →	1.02	0.01	3rd Amend. →	1.02	0.01
97		1.13	0.05		1.11	0.05
98		1.44	0.16		1.46	0.16
99		1.77	0.25		1.82	0.26
100		2.47	0.39*		2.83	0.45*
101		4.03	0.61*		5.57	0.75*
102		9.31	0.97*		17.23	1.24*

[a] values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

[b] sl = sample lost

	Replicate 1	Replicate 2
1st Amend.	$k = 0.068$, $R^2 = 0.93$	$k = 0.087$, $R^2 = 0.90$
2nd Amend.	$k = 0.368$, $R^2 = 0.98$	$k = 0.564$, $R^2 = 0.94$
3rd Amend.	$k = 0.668$, $R^2 = 0.98$	$k = 0.910$, $R^2 = 0.98$

Table 6. (Continued)

Incubation Time (hr)	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
50	3.43×10^{6}*[b]		1.61×10^{6}*	
54	7.83*	9.79 ± 5.13	4.50*	7.55 ± 5.51
60	13.70*		10.30*	
64	14.20*		13.80*	
74	54.10*		19.70*	
78	26.60*	43.70 ± 31.74	30.90*	34.65 ± 12.36
84	11.00*		39.20*	
88	83.10*		48.80*	
98	37.80*		37.00*	
102	184.00*	86.45 ± 69.36	64.00*	61.0 ± 22.65
108	88.00*		82.00*	
112	36.00*		401.00	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters organism^{-1} hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	$K = 6.96 \times 10^{-9}$	$K = 1.15 \times 10^{-8}$
2nd Amend.	$K = 8.43 \times 10^{-9}$	$K = 1.63 \times 10^{-8}$
3rd Amend.	$K = 7.73 \times 10^{-9}$	$K = 1.49 \times 10^{-8}$

Table 7. Sequential amendment study, 100 ppb p-cresol, Yakima River - Horn Rapids Park boat ramp (4-22-83)

Incubation Time (hr)		Replicate 1 Init./Res.	Log		Replicate 2 Init./Res.	Log
24		1.54	0.19*[a]		1.47	0.17*
25		1.25	0.10*		1.27	0.10*
26		1.68	0.23*		1.94	0.29*
27		1.62	0.21*		1.84	0.26*
28		2.20	0.34*		1.78	0.25*
29		2.51	0.40*		1.99	0.30*
30		2.35	0.37*		2.16	0.33*
31		2.43	0.39*		2.22	0.35*
32		3.09	0.49*		2.88	0.46*
34		5.00	0.70*		2.63	0.42*
36		7.07	0.85*		2.93	0.47*
38		11.22	1.05*		7.05	0.85*
40		73.68	1.87*		10.77	1.03*
48	2nd Amend. →	1.04	0.02	2nd Amend. →	1.07	0.03
49		1.14	0.06		1.17	0.07
50		1.22	0.09		1.26	0.10
51		1.36	0.13		1.38	0.14
52		1.54	0.19		1.47	0.17
53		1.65	0.22		1.69	0.23
54		2.00	0.30		2.01	0.30
55		2.46	0.39		2.78	0.44
56		2.34	0.37*		3.10	0.49*
58		5.38	0.73*		6.93	0.84*
60		23.11	1.36*		74.21	1.87*
72	3rd Amend. →	0.98	-0.01	3rd Amend. →	1.08	0.03
73		1.02	0.01		1.14	0.06
74		1.14	0.06		1.27	0.10
75		1.28	0.11		1.46	0.16
76		1.44	0.16		1.67	0.22
77		1.82	0.26		2.22	0.35
78		2.30	0.36*		3.13	0.50*
79		3.17	0.50*		4.79	0.68*
80		5.32	0.73*		11.44	1.06*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	$k = 0.201$, $R^2 = 0.83$	$k = 0.110$, $R^2 = 0.85$
2nd Amend.	$k = 0.570$, $R^2 = 0.98$	$k = 0.795$, $R^2 = 0.93$
3rd Amend.	$k = 0.426$, $R^2 = 0.98$	$k = 0.645$, $R^2 = 0.96$

Table 7. (Continued)

Incubation Time (hr)	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
26	1.35×10^{6}*[b]		1.60×10^{6}*	
30	6.53*		1.82*	
36	3.10*	3.71 ± 2.15	2.13*	1.84 ± 0.22
40	3.87*		1.79*	
50	1.87*		1.75*	
54	8.67*	5.24 ± 3.09	4.17*	3.86 ± 2.96
60	3.55*		1.58*	
64	6.87*		7.93*	
74	1.66*		2.20*	
78	5.70*	5.23 ± 2.52	2.23*	3.56 ± 2.33
84	5.97*		2.77*	
88	7.57*		7.03*	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters organism^{-1} hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	$K = 5.42 \times 10^{-8}$	$K = 5.97 \times 10^{-8}$
2nd Amend.	$K = 10.90 \times 10^{-8}$	$K = 20.50 \times 10^{-8}$
3rd Amend.	$K = 8.16 \times 10^{-8}$	$K = 18.20 \times 10^{-8}$

For p-cresol, pseudo first-order rate constants ranged from 0.041 to 0.790 per hour. However, much of the variation in these values could be accounted for in the range of bacterial populations (1.84×10^6 to 4.08×10^8 cfu/L) used to determine second-order rate constants. Examination of the calculated second-order rate constants suggested they could be divided into the two groups listed in Table 4. Each of the constants in Group II, when examined individually against Group I by a Q test, were significantly different at a minimum confidence level of 0.90. The first group, for which second-order constants range from 1.06×10^{-9} to 1.15×10^{-8} L organism^{-1} hr^{-1} and had a mean value of $(4.59 \pm 3.00) \times 10^{-9}$ L organism^{-1} hr^{-1}, comprises 70% of the calculated constants. The second group of constants, which ranged in value from 1.87×10^{-8} to 5.97×10^{-8} L organism^{-1} hr^{-1} and had a mean value of $(3.49 \pm 1.74) \times 10^{-8}$ L organism^{-1} hr^{-1}, contained the remaining 30% of the constants. It is noteworthy that the range in second-order constants of each group was reduced in comparison to the variability in the pseudo first-order constants (Table 5). This was especially true for the Group I constants where the second-order constants varied ~10-fold and the first-order constants varied ~20-fold.

Quinoline and α-Naphthol

Analysis of initial quinoline and α-naphthol transformation data indicated that these compounds were only transformed after a lengthy and variable lag period. Because of this, the development of transformation data was uncertain unless extensive sampling schemes were employed. This was unlike p-cresol, where the lag period could be predicted with some certainty and experimental parameters could be adjusted for recovery of sufficient data for the determination of second-order rate constants (see Appendix B). To facilitate the determination of pseudo first-order and second-order rate constants for quinoline and α-naphthol, the use of sequential amendments in the design of experiments was investigated. In these experiments each new substrate amendment was added after the concentration of the previous amendment had fallen to a less than detectable value. This procedure allowed the bacterial population to adapt to the test substrate and thus reduced the total number of samples required and defined, with greater efficiency, the time period when transformation of the test compound was optimal.

This procedure was first examined with p-cresol for which adaptation generally occurred within 24 to 48 hr. These experiments were conducted at two substrate concentrations (1000 ppb and 100 ppb) and each water sample was reamended twice. At both concentrations, a substantial lag period was observed before a decrease in p-cresol concentration was measured (Tables 6 and 7; Figures 1 and 2) whereas

FIGURE 1. Sequential amendment study, 1000 ppb p-cresol, Yakima River - Horn Rapids Park boat ramp (4-22-83)

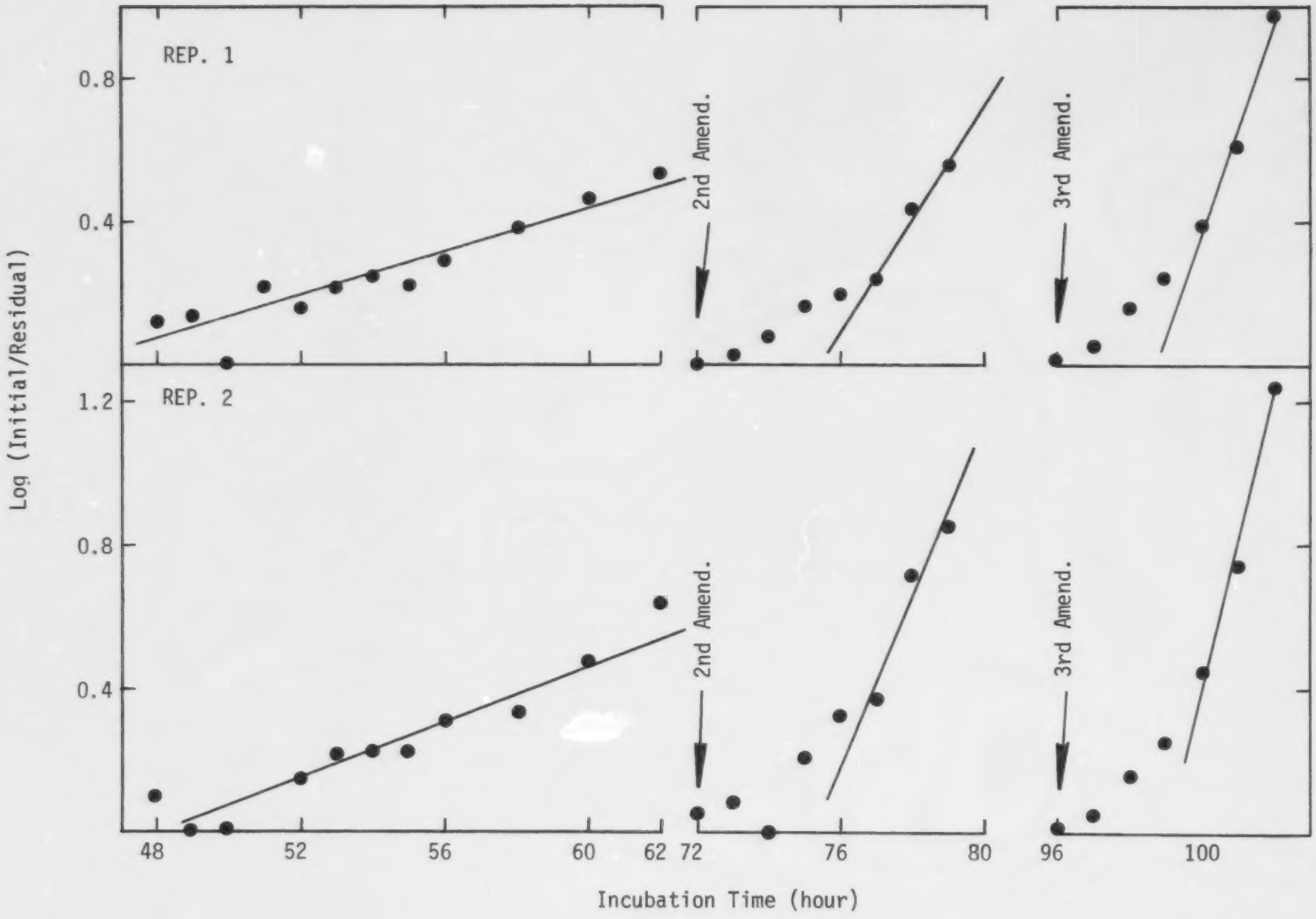
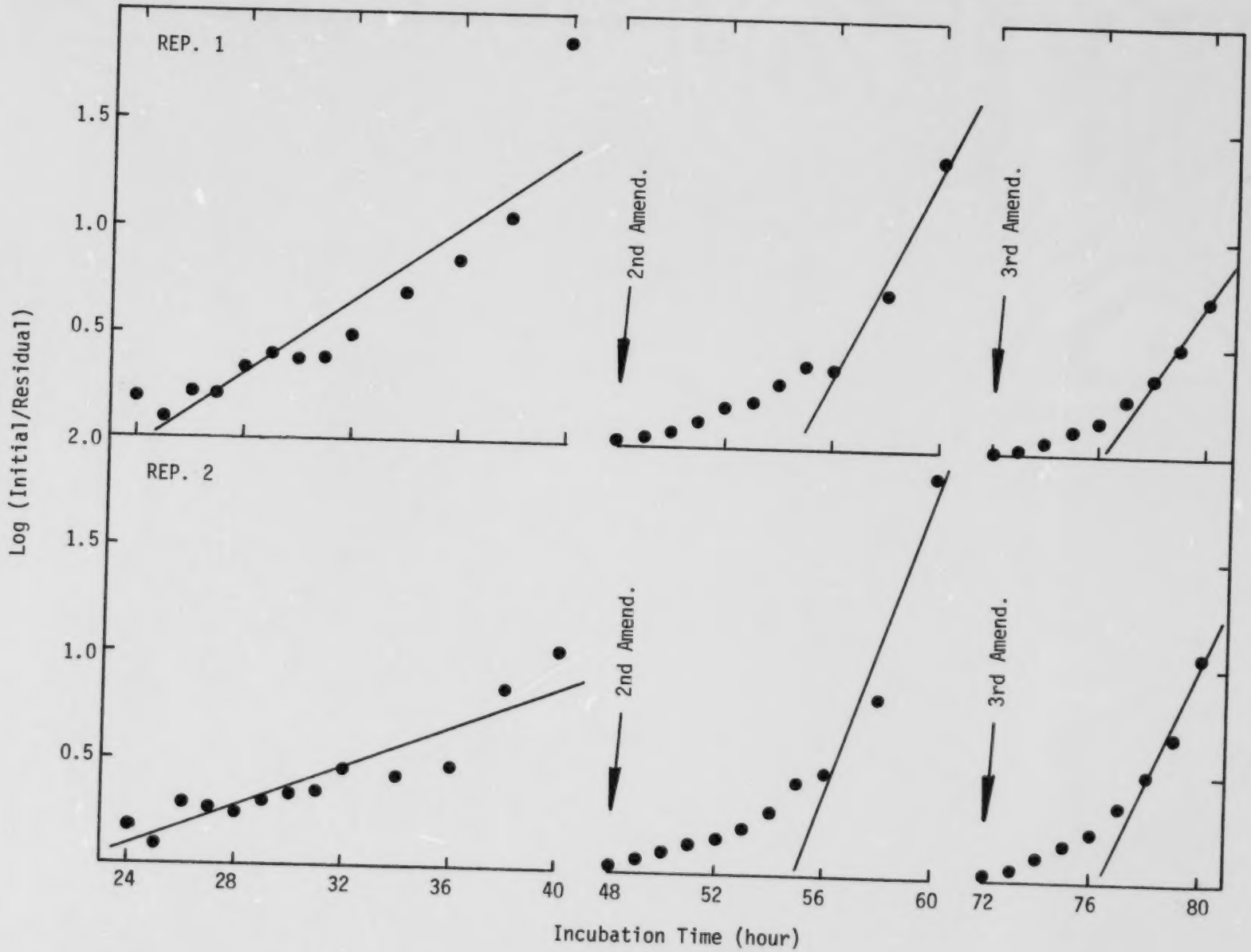


FIGURE 2. Sequential amendment study, 100 ppb p-cresol, Yakima River - Horn Rapids Park boat ramp (4-22-83)



after the second and third amendments p-cresol was transformed without a lag period, suggesting that the microbial population was adapted. Comparison of the second-order rate constants over the four water samples (Table 8) and the three amendments indicated a marked dissimilarily in constants compared between waters and a marked similarity in constants derived from a given water sample. The general agreement between rate constants derived from a single water sample indicated the potential utility of using multiple amendments to estimate second-order rate constants for compounds having extended and variable lag periods.

Nine waters were examined for their ability to transform quinoline using sequential amendment experiments. The data collected from these studies are summarized in Appendix C. In all cases a lag period that varied from 3 to 10 days occurred before the quinoline concentration decreased. Complete transformation of the quinoline generally required an additional 24 to 48 hours. Quinoline added to these waters in subsequent amendments was also generally transformed within 24 to 48 hour period, which included a short lag phase (2 to 4 hr) for some waters.

For all but one water, data collected from the second amendment was used to calculate the pseudo first-order rate constants. Only with the Walla Walla River sample (5-2-83) was the data from the third amendment used. Where possible, constants were determined from the linear portion of the transformation phase of the first-order plot. When linearity could not be readily identified a rate constant was estimated from the last three data points.

Table 8. Summary of second-order rate constants for p-cresol from sequential amendment studies

	Second-Order Rate Constant			
Substrate Amendment	1000 ppb p-Cresol		100 ppb p-Cresol	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1st	6.96×10^{-9}	1.15×10^{-8}	5.42×10^{-8}	5.97×10^{-8}
2nd	8.43×10^{-9}	1.63×10^{-8}	10.90×10^{-8}	20.50×10^{-8}
3rd	7.73×10^{-9}	1.49×10^{-8}	8.16×10^{-8}	18.20×10^{-8}

For quinoline, pseudo first-order rate constants ranged from 0.0085 to 1.57 per hour. Calculation of the second-order rate constants from the average bacterial concentration in each case yielded constants that fell into one of three groups (Table 9). Group I, which clustered around a mean value of $(2.00 \pm 1.16) \times 10^{-10}$ L organism^{-1} hr^{-1}, ranged from 1.10×10^{-10} to 4.02×10^{-10} L organism^{-1} hr^{-1}. Group II ranged from 1.38×10^{-9} to 5.05×10^{-9} L organism^{-1} hr^{-1} and clustered around a mean of $(3.51 \pm 1.40) \times 10^{-9}$ L organism^{-1} hr^{-1}. Group III only contained two constants with a mean of $(3.51 \pm 0.41) \times 10^{-7}$ L organism^{-1} hr^{-1}. Each of the constants in Group II, when examined individually against Group I by a Q test, were significantly different at a minimum confidence level of 0.90. A similar difference was observed for Groups II and III. Much of the variation observed in the pseudo first-order rate constants could be accounted for in the range of bacterial populations (2.5×10^6 to 6.1×10^9 cfu/L) used to determine the second-order constants. This was most evident for Groups I and II (Table 10) where the first-order constants ranged 124 to 185-fold and the second-order constants ranged 1.18 to 3.7-fold.

Sequential amendment experiments were used to examine the microbiological transformation of α-naphthol over a total of six waters. A summary of the data obtained in these experiments can be found in Appendix D. For α-naphthol, both the initial lag period and the time for complete transformation to occur following the lag varied among the six waters examined. The lag period ranged from 2 to 8 days; the time required for complete transformation ranged from 1 to 13 days. The transformation of α-naphthol added in succeeding amendments generally followed a shorter lag period (1 to 2 days), however, the time over which transformation occurred was again variable and ranged from 1 to 16 days.

Pseudo first-order rate constants for α-naphthol were determined using transformation data collected following the initial amendment, the second amendment, and for one water after the third amendment (Table 11). Where possible, first-order constants were determined from the linear portion of the first-order plot. Rate constants were determined from the last three data points when linearity could not be identified.

Pseudo first-order rate constants for α-naphthol varied over a wide range with the greatest variability occurring in the values obtained for the second and third substrate amendments (0.004 to 0.386 per hour). The variability in first-order constants determined from the initial α-naphthol amendments (Group III) was much less (0.006 to 0.026 per hr). Second-order constants determined from initial amendment data ranged

Table 9. Second-order rate constants for quinoline

Water Sample	Initial Conc. (ppm)	Replicate	1st Amend. K	2nd Amend. K	3rd Amend. K
			- - liters organism^{-1} hr^{-1} - -		
Group I					
Snake River - Hood Park Boat Ramp (8-1-83)	1.0	1	ns[a]	ns	nd[b]
	1.0	2	ns	1.45×10^{-10}	nd
Columbia River - Richland "Wye" Marina (8-1-83)	1.0	1	ns	4.02×10^{-10}	nd
Walla Walla River Madame Dorion Park (9-7-83)	1.0	1	ns	1.79×10^{-10}	nd
	1.0	2	ns	1.10×10^{-10}	nd
331 Bldg. Fish Pond (9-7-83)	1.0	1	ns	1.64×10^{-10}	nd
				MEAN[c] $(2.00 \pm 1.16) \times 10^{-10}$	
Group II					
Walla Walla River Madame Dorion Park (5-2-83)	1.0	1	ns	ns	4.37×10^{-9}
	1.0	2	ns	ns	3.35×10^{-9}
Columbia River - Richland "Wye" Marina (8-1-83)	1.0	2	ns	5.05×10^{-9}	nd
331 Bldg. Fish Pond (9-7-83)	1.0	2	ns	3.36×10^{-9}	nd
Yakima River - Horn Rapids Park Boat Ramp (9-7-83)	1.0	1	ns	1.38×10^{-9}	nd
				MEAN[d] $(3.51 \pm 1.40) \times 10^{-9}$	
Group III					
Yakima River - Horn Rapids Park Boat Ramp (8-1-83)	1.0	1	ns	3.22×10^{-7}	nd
	1.0	2	ns	3.80×10^{-7}	nd
				MEAN[e] $(3.51 \pm 0.41) \times 10^{-7}$	

[a] ns = not sufficient data to determine rate constant

[b] nd = third amendment not done

c = calculated from 2nd amend. K values

d = calculated from 2nd and 3rd amend. K values
e = calculated from 2nd amend. K values

Table 10. Comparison of pseudo first-order and second-order rate constants for quinoline

	No. of Constants Compared	Range of Constants	
		Pseudo First-Order (hr^{-1})	Second-Order (liters $organism^{-1}$ hr^{-1})
Group I	(5)	0.037 to 0.668 (185)[a]	1.10×10^{-10} to 4.02×10^{-10} (3.65)
Group II	(5)	0.0085 to 1.57 (184.71)	1.38×10^{-9} to 5.05×10^{-9} (3.69)
Group III	(2)	0.570 to 0.708 (1.24)	3.22×10^{-7} to 3.80×10^{-7} (1.18)

[a]ratio of highest and lowest values

Table 11. Summary of second-order rate constants for α-naphthol

Water Sample	Initial Conc. (ppm)	Replicate	1st Amend. K	2nd Amend. K	3rd Amend. K
			- - - - liters organism^{-1} hr^{-1} - - - -		
Group I					
Yakima River - Horn Rapids Park Boat Ramp (5-2-83)	1.0	2	3.73×10^{-9}	4.79×10^{-11}	nd[b]
Snake River - Hood Park Fish Pond (5-2-83)	1.0	1	3.17×10^{-9}	8.77×10^{-11}	nd
	1.0	2	5.71×10^{-9}	7.37×10^{-11}	nd
Columbia River - Richland "Wye" Marina (8-19-83)	1.0	2	ns[a]	7.34×10^{-11}	nd
				MEAN[c] $(7.07 \pm 1.66) \times 10^{-11}$	
Group II					
Yakima River - Horn Rapids Park Boat Ramp (5-2-83)	1.0	1	2.34×10^{-9}	5.94×10^{-10}	nd
Walla Walla River Madame Dorion Park (5-2-83)	1.0	1	4.22×10^{-9}	6.69×10^{-10}	nd
	1.0	2	8.35×10^{-10}	1.26×10^{-9}	2.84×10^{-10}
Columbia River - Richland "Wye" Marina (8-19-83)	1.0	1	ns	7.81×10^{-10}	nd
331 Bldg. Fish Pond (8-19-83)	1.0	1	ns	2.34×10^{-10}	nd
	1.0	2	ns	1.58×10^{-10}	nd
				MEAN[d] $(5.69 \pm 3.86) \times 10^{-10}$	
Group III			MEAN[e] $(3.33 \pm 1.66) \times 10^{-9}$		
Group II plus Group III			MEAN $(1.84 \pm 1.81) \times 10^{-9}$		

[a] ns = not sufficient data to determine rate constants

[b] nd = third amendment not done

c = calculated from 2nd amend. K values

d = calculated from 2nd and 3rd amend. K values

e = calculated from all 1st amend. K values

from 8.35×10^{-10} to 5.71×10^{-9} L organism^{-1} hr^{-1} with a mean of $(3.33 \pm 1.66) \times 10^{-9}$ L organism^{-1} hr^{-1} (Tables 11 and 12). Examination of the data from the second and third amendments suggested that the calculated second-order constants could be subdivided into two groups; each constant in the second group being significantly different from the first group at a minimum confidence level of 0.90 when examined by a Q test. However, Group II constants could not be distinguished from the Group III constants determined from the initial α-naphthol amendments thus these two groups were combined (Table 11) to yield a mean value of $(1.84 \pm 1.81) \times 10^{-9}$ L organism^{-1} hr^{-1}.

Table 12. Comparison of pseudo first-order and second-order rate constants for α-naphthol

	No. of Constants Compared	Range of Constants	
		Pseudo First-Order (hr^{-1})	Second-Order (liters organism^{-1} hr^{-1})
Group I	(4)	0.0040 to 0.015	4.79×10^{-11} to 8.77×10^{-11}
		(3.75)[a]	(1.83)
Group II	(7)	0.0057 to 0.386	1.58×10^{-10} to 1.26×10^{-9}
		(67.72)	(7.97)
Group III	(6)	0.0055 to 0.026	8.35×10^{-10} to 5.71×10^{-9}
		(4.73)	(6.84)
Group II plus Group III	(13)	0.0055 to 0.386	1.58×10^{-10} to 5.71×10^{-9}
		(70.18)	(36.14)

[a]ratio of highest and lowest values

Second-order rate constants for Group I ranged from 4.79×10^{-11} to 8.77×10^{-11} L organism^{-1} hr^{-1} with a mean of $(7.07 \pm 1.66) \times 10^{-11}$ L organism^{-1} hr^{-1}. For the second group, the constants ranged from 1.58×10^{-10} to 1.26×10^{-9} L organism^{-1} hr^{-1} with a mean of $(6.16 \pm 4.00) \times 10^{-10}$ L organism^{-1} hr^{-1}. A significant portion of the variability in the first-order constants (Table 12) determined from the second and third substrate amendments could be accounted for in the range of measured bacterial populations (1.1×10^{6} to 1.9×10^{9} cfu/L) used to calculate second-order rate constants for Group II. For Group II the first-order constants ranged 680-fold and the second-order constants ranged 8.0-fold, whereas for Group I the ranges were 3.7 and 1.8-fold.

Malathion

Studies designed to provide data to calculate second-order rate constants for the transformation of malathion in river water samples were unsuccessful because of the low bacterial populations found in the waters sampled. Efforts to increase the microbial populations by preincubation in the presence of small quantities of nutrient broth or using the supernatant solution from sediment suspensions were also unsuccessful. Therefore, no data are reported.

DISCUSSION

The microbiological transformation rates of four organic compounds, 2,4-DBE, p-cresol, α-naphthol and quinoline added to natural water samples were examined in laboratory experiments. Graphical analysis of the data with first-order log plots indicated that the transformation of these compounds occurred in two phases (Appendixes B, C and D). The initial phase consisted of a lag period during which no measurable decrease in compound concentration could be detected. Three of the compounds p-cresol, α-naphthol and quinoline were only transformed after such a lag phase. The transformation of 2,4-DBE, however, occurred immediately upon addition of the compound to sample waters. The lag period was followed by a transformation phase where the detectable decrease in compound concentration could be described by a pseudo first-order rate equation for which a pseudo first-order rate constant could be determined. For α-naphthol and quinoline, transformation occurred after a variable and generally lengthy lag period which interfered with the collection of sufficient data to determine pseudo first-order constants. The use of sequential amendment studies was examined to circumvent this problem.

The variability in first-order constants observed for the different compounds ranged from a low of 13.6-fold for 2,4-DBE to a high of 185-fold for quinoline. Much of the variability could be accounted for in the range in the average bacterial populations, measured during the transformation phase, which were used to calculate second-order rate constants. The second-order rate constants could be clustered into groups that were statistically different (Tables 2, 4, 9 and 11). The variability of second-order constants within these groups ranged from 1.18-fold for quinoline (Group III) to 36.14 for α-naphthol (Group II plus Group III) whereas the first-order constants ranged from 1.24 for quinoline (Group III) to 184.7 for quinoline (Group II). Within all but two groups, the range in first-order rate constants was greater than the range in second-order constants. For the compounds p-cresol, α-naphthol and quinoline the clustering of second-order constants suggests that the number of microbiological communities that can adapt in the laboratory for the transformation or degradation of these compounds during the lag phase is limited. For 2,4-DBE, where a lag was not observed and most of the variability in second-order rate constants occurred from the analysis of a single water (Table 2). The clustering of constants in groups could not be explained by adaptation to 2,4-DBE. Alternatively, these results would suggest that microbial communities with significantly different transformation rates for 2,4-DBE can develop in the natural environment independent of its presence. However, the less

likely explanation that a community having greater transformation activity developed during the equilibration of waters to laboratory conditions before addition of 2,4-DBE cannot be ignored.

Comparison of the second-order rate constants for 2,4-DBE and p-cresol (Table 13) reported herein and the second-order constants reported previously by Paris et al., 1981 and Paris et al., 1983, indicated that although the mean values were markedly different the standard deviations were remarkably close in all cases. Either of two possible explanations can account for the differences in these values; there is a systematic difference between laboratory procedures or there were significant differences in the microbial populations in waters sampled by each of the laboratories and the differences are real. The first possibility is supported by the observation that the differences observed for our Group I constants, which represent the major fraction of the constants determined, and the Paris constants were similar for both compounds, 29.8 ± 19.0-fold for 2,4-DBE and 17.0 ± 13.8-fold for p-cresol. Of the two parameters measured in these experiments, an accurate measure of the bacterial populations was more difficult and could possibly account for the differences observed. The second possibility is supported by our observation that second-order rate constants can be clustered into distinct groups greater than an order of magnitude apart and previous literature reports (Burns, 1982) that values for second-order rate constants can vary up to ten-fold depending on the source of the microorganisms. Clarification of these differences

Table 13. Comparison of second-order rate constants

	2,4-DBE (liters organism^{-1} hr^{-1})	p-Cresol (liters organism^{-1} hr^{-1})
Paris et al., 1981 and 1983	$(5.4 \pm 2.7) \times 10^{-10}$	$(2.7 \pm 1.3) \times 10^{-10}$
Our work		
- Group I	$(1.61 \pm 0.64) \times 10^{-8}$	$(4.59 \pm 3.01) \times 10^{-9}$
- Group II	$(2.07 \pm 1.17) \times 10^{-7}$	$(3.24 \pm 1.50) \times 10^{-8}$

would require that both laboratories conduct a limited study with the same series of water samples to detect systematic differences in laboratory procedures.

Because a variable and sometimes lengthy lag period preceded the transformation of quinoline and α-naphthol, second-order rate constants were determined from sequential amendment experiments. This technique has proved to be a potentially useful tool. For p-cresol second-order rate constants calculated from second and third amendment, data (Table 8) were generally equivalent to or greater than a factor of three different from the first amendment constants. Similar observations were made for α-naphthol where the mean second-order constants determined from first and second amendment data were not significantly different (Table 11).

Before a detectable decrease in compound concentration could be measured for three of the four compounds examined, a significant lag period was observed. This lag could have importance in the approach used in computer modeling of compounds that behave in this manner. Several possible situations can be considered. The first situation assumes that the lag phase is not significant over the total time required for degradation of the compound because of continuous input of the contaminant to the environment and is simply modeled using the second-order rate equation. This approach to modeling would also be used if no lag period was anticipated as would be expected for compounds like 2,4-DBE. The second situation, which is illustrated by the results for p-cresol (Appendix B) and α-naphthol (Appendix D), assumes that the lag phase and the transformation phase are of equal importance. This situation may occur when release of the compound to the environment is a single event or when multiple releases occur with sufficient time between events for the microbial population to return to a former state. The modeling of such releases would require that equal consideration be given to both phases. In the final situation the lag phase is considered to be dominant. Such situations would occur from single release events where modeling efforts would emphasize the lag phase. These situations may correlate with specific compounds; however, it is more likely that they will correlate to concentration ranges which will probably vary from one compound to the next. An effect of concentration on the lag phase has been observed herein for p-cresol (Appendix B) and by Paris et al., 1982, for the transformation of p-cyanophenol.

One of the primary interests in developing site independent second-order rate constants has been their use in exposure assessment modeling. The reproducibility of these constants for a number of compounds observed in our work and in that of others (Paris et al.,

1981, and Paris et al., 1983) emphasizes their utility in exposure assessment. However, the role of microbiological transformation or degradation of xenobiotics cannot be limited only to second-order constants. The potential importance of the lag phase (adaptation period) is emphasized in this report and the observation that second-order rate constants fall into groups suggests that the rate of transformation of xenobiotics in the environment may require a probability distribution function, i.e., transformation models may need to have a random variable format. The conservative alternative would be to simply use the smallest second-order rate constant determined for a given compound. Although more data are needed with different sites and different compounds, these results do offer significant encouragement for prediction of microbial transformation rates in natural systems.

RECOMMENDATIONS

A continued research effort in this area, which is a "kinetic approach" to the predictive modeling of microbiological transformations in the environment, should address the areas of adaptation kinetics, reaction kinetics and quantitation of biomass. Biomass has been included because of its importance in extrapolating from basic research models to widely applicable predictive models that are normally constructed to use readily available or easily attainable data. For example, a compound specific biomass measure can now be obtained by using a most probable number technique for enumerating microorganisms that incorporates ^{14}C-labeled substrates. In time this procedure may displace the standard pour plate methods currently being used.

An extended research effort in these areas should contain at least the following set of tasks and subtasks.

Task 1. Identify adaptation phenomena for different compound classes at different concentrations.

Subtask 1. Classify compound as to 1, 2 and 3 below.

1. No adaptation.

2. Adaptation is approximately equal to half the time required to degrade the compound.

3. Adaptations are markedly longer than time required for degradation.

Subtask 2. Define the kinetics of the adaptation process (growth, adjustment to compound, induction, etc.)

Subtask 3. Define computer inputs and mathematical expression for different adaptation processes, e.g., Monod kinetics which require input of binding constants and specific growth rates.

Task 2. Identification of specific transformation kinetic processes.

Subtask 1. Characterize compound classes as to pseudo first-order, pseudo second-order Monod and Michaelis-Menten kinetics.

Subtask 2. Define computer inputs and mathematical expressions for different kinetic processes.

Task 3. Develop site independent biomass measures.

Subtask 1. Develop compound specific biomass measures (e.g., MPN).

Subtask 2. Develop general biomass measures which relate to specific measures (e.g., cfu).

Where second-order rate equations are applicable "the kinetic approach" has rested on the ability of laboratory studies to determine an average second-order rate constant, using a number of different waters with different microbial populations. The average rate constants could then be used to predict degradation rates at other sites if the microbial population and organic substrate concentrations are known.

This approach can also be used when the degradation rate is described by alternative mathematical expressions. For example, if the Michaelis-Menten equation (Cornish-Bowden, 1976) were used, one would simply determine the average values for K_m, V_{max}, and the bacterial population of a number of waters and then use these average values to estimate degradation rates in other waters where the substrate concentration and bacterial populations are known. The bacterial population in this case is used to set the value of V_{max} from an average specific activity term (sp. act. = V_{max}/bacterial population) determined in the initial evaluation studies. Similar examples can be derived from other mathematical expressions of the degradation rate.

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APPENDIX A

SECOND-ORDER RATE CONSTANTS FOR 2,4-DBE

Table A-1. 100 ppb 2,4-DBE, 331 Building Fish Pond (1-31-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
4	1.17	0.07*[a]	1.21	0.08*
6	1.04	0.02*	1.26	0.10*
10	1.17	0.07*	1.15	0.06*
12	1.14	0.06*	1.24	0.09*
14	1.36	0.13*	1.39	0.14*
16.75	1.50	0.17*	1.40	0.15*
24	2.76	0.44*	2.50	0.40*
28	2.44	0.39*	2.53	0.40*
32	3.16	0.50*	2.75	0.44*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.042$, $R^2 = 0.91$	$k = 0.035$, $R^2 = 0.89$

Time (hr)	Bacterial Concentration, cfu[a]/liter	
	Replicate 1	Replicate 2
5.25	0.73×10^6*[b]	0.87×10^6*
11.25	0.90* $(1.78 \pm 1.12) \times 10^6$	1.37* $(2.26 \pm 1.35) \times 10^6$
16.75	2.70*	3.10*
33.25	2.80*	3.70*

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 2.34 \times 10^{-8}$	$K = 1.55 \times 10^{-8}$

Figure A-1. 100 ppb 2,4-DBE, 331 Building Fish Pond (1-31-83)

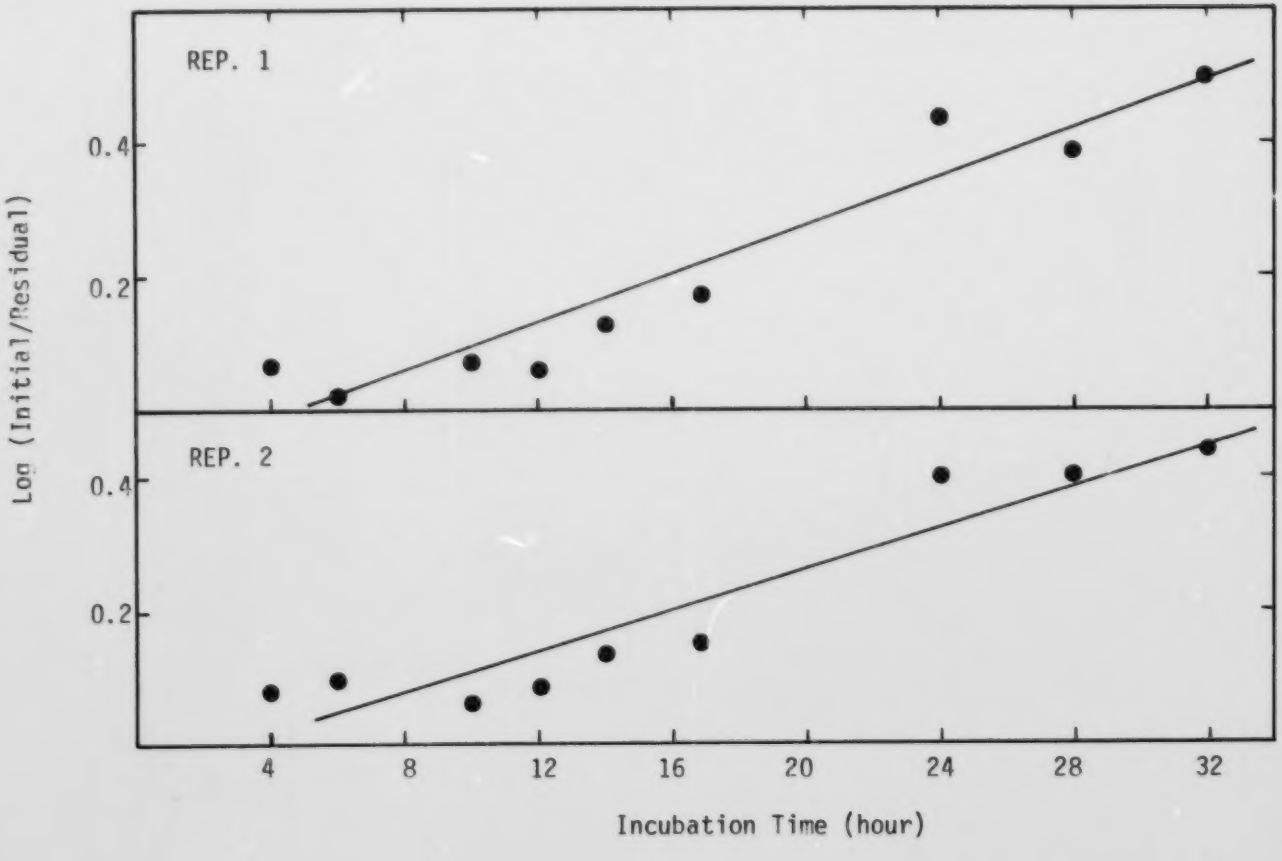


Table A-2. 100 ppb 2,4-DBE, 331 Building Fish Pond (2-8-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
2	1.06	0.02*	0.94	-0.03
4	1.10	0.04*	1.12	0.05*
6	1.13	0.05*	1.19	0.07*
8	1.08	0.03*	1.00	-9.4×10^{-4}
10	1.12	0.05*	1.03	0.01*
12	1.04	0.02*	1.22	0.08*
14	1.40	0.15*	1.41	0.15*
16	1.48	0.17*	1.48	0.17*
18	1.70	0.23*	1.64	0.21*
24	2.41	0.38*	2.70	0.43*
28	1.93	0.29*	3.17	0.50*
32	2.27	0.36*	3.13	0.50*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.0304$, $R^2 = 0.86$	$k = 0.045$, $R^2 = 0.91$

Time (hr)	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
6	0.83×10^{6}*[b]		3.83×10^{6}	
11	1.67*		3.23	
18	1.17*	$(1.19 \pm 0.30) \times 10^{6}$	4.97	$(5.28 \pm 2.06) \times 10^{6}$
26	0.96*		4.80	
31	1.38*		9.07	
35	1.12*		5.80	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 2.55 \times 10^{-8}$	$K = 8.54 \times 10^{-9}$

Figure A-2. 100 ppb 2,4-DBE, 331 Building Fish Pond (2-8-83)

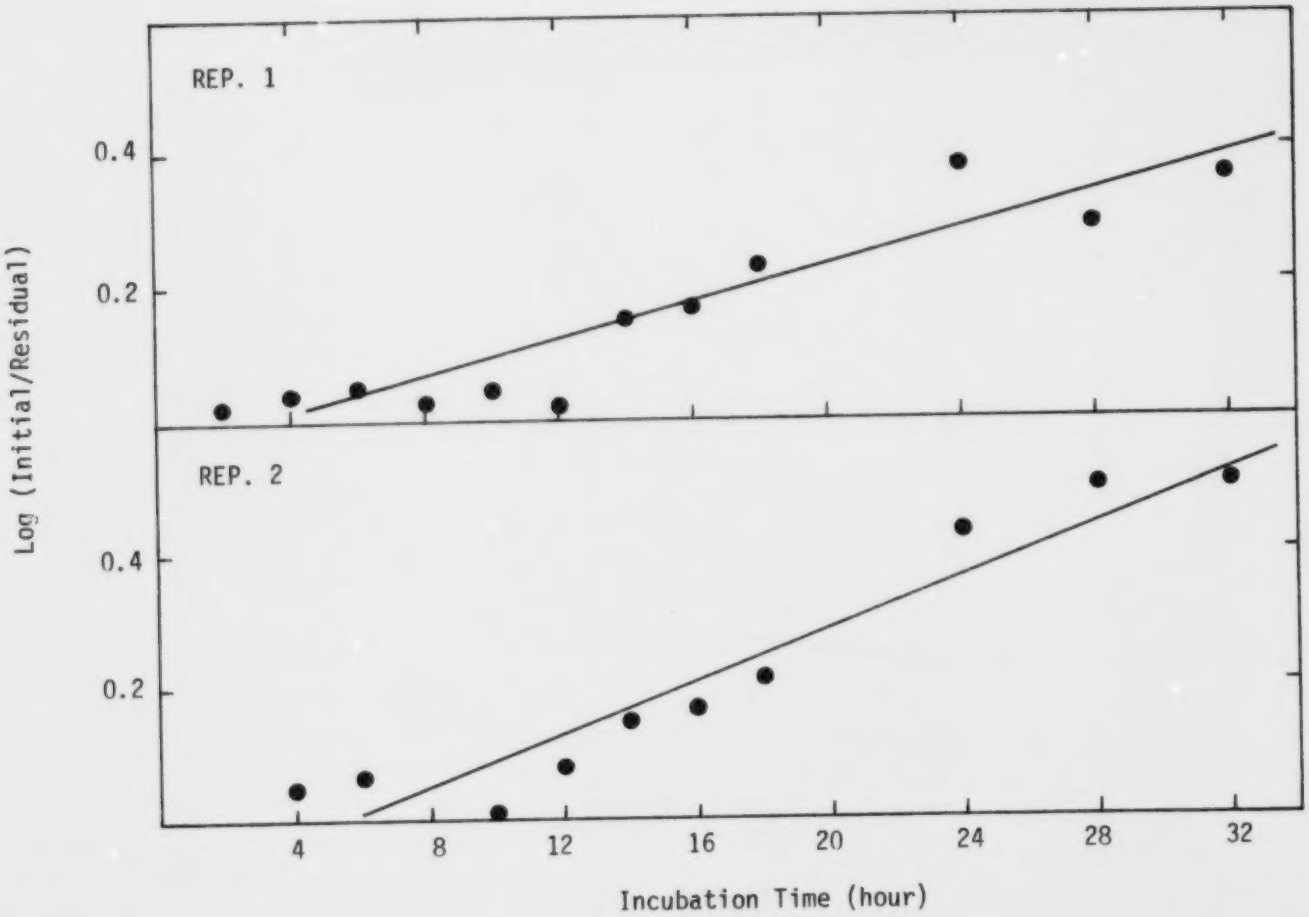


Table A-3. 100 ppb 2,4-DBE, Columbia River - Richland "Wye" Marina (2-14-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
0	1.83	0.26*	1.19	0.08*
4	2.02	0.31*	1.16	0.07*
8	5.80	0.76*	5.8	0.76*
12	19.04	1.28*	12.79	1.11*
16	-	-	43.96	1.64*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.202$, $R^2 = 0.91$	$k = 0.240$, $R^2 = 0.95$

Time (hr)	Bacterial Concentration, cfu[a]/liter	
	Replicate 1	Replicate 2
2	9.60×10^6*[b]	1.90×10^6*
12	9.20* $(9.13 \pm 0.50) \times 10^6$	3.50* $(3.20 \pm 1.18) \times 10^6$
16	8.60*	4.20*

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 2.21 \times 10^{-8}$	$K = 7.48 \times 10^{-8}$

Figure A-3. 100 ppb 2,4-DBE, Columbia River - Richland "Wye" Marina (2-14-83)

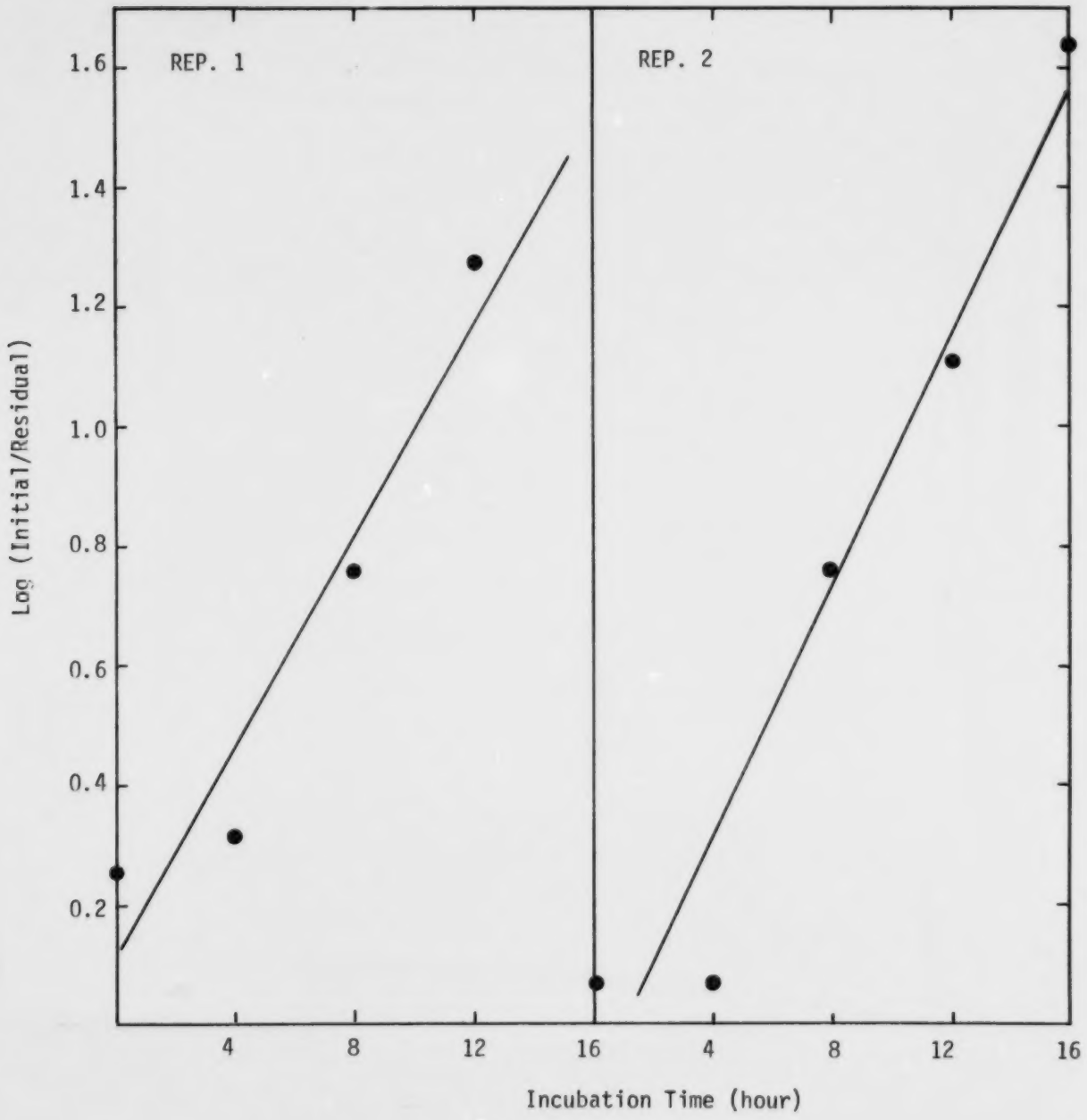


Table A-4. 100 ppb 2,4-DBE, Columbia River - Richland "Wye" Marina (2-28-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
2.5	1.20	0.08*	1.28	0.11*
3	1.08	0.03*	1.21	0.08*
3.5	1.21	0.08*	1.14	0.06*
4	1.74	0.24	1.58	0.20
4.5	1.15	0.06*	1.18	0.07*
5	1.02	0.01	0.94	-0.03
6	1.48	0.17*	1.47	0.17*
7	1.35	0.13*	1.35	0.13*
9.5	1.01	0.01	1.33	0.12*
10	1.62	0.21*	1.57	0.19*
12	1.47	0.17*	1.65	0.22*
20	2.88	0.46*	3.07	0.49*
24	3.37	0.53*	3.58	0.55*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.051$, $R^2 = 0.95$	$k = 0.053$, $R^2 = 0.93$

Time (hr)	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
0	1.31×10^6		1.10×10^6	
4	1.83*		2.32*	
9	4.30*	$(4.06 \pm 1.75) \times 10^6$	4.50*	$(4.93 \pm 1.97) \times 10^6$
12.5	4.00*		6.30*	
24	6.10*		6.60*	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 1.26 \times 10^{-8}$	$K = 1.07 \times 10^{-8}$

Figure A-4. 100 ppb 2,4-DBE, Columbia River - Richland "Wye" Marina (2-28-83)

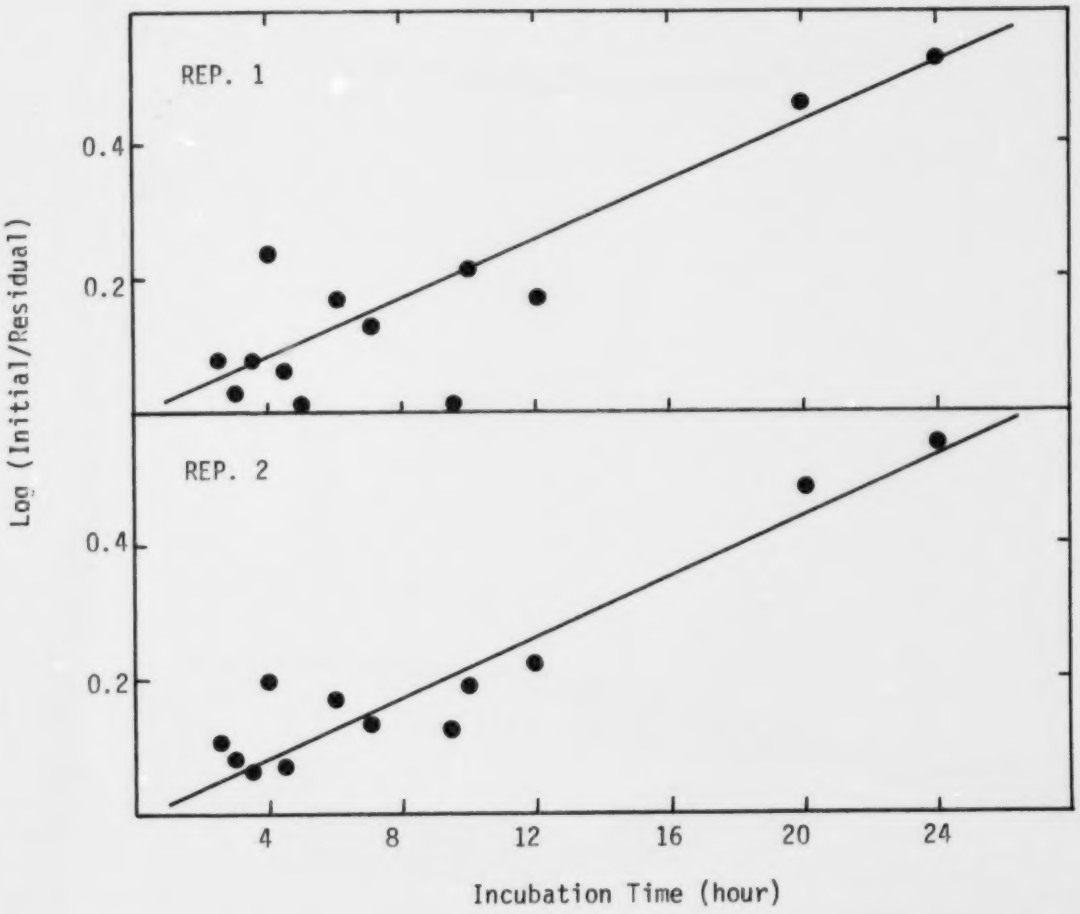


Table A-5. 100 ppb 2,4-DBE, Yakima River - Horn Rapids Park Boat Ramp (3-1-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
1	1.13	0.05*	1.35	0.13*
2	1.37	0.14*	1.37	0.14*
3	1.72	0.24*	1.93	0.29*
3.5	1.78	0.25*	2.03	0.31*
4	1.98	0.30*	2.07	0.32*
4.5	1.62	0.21	2.14	0.33*
5	2.87	0.46*	3.26	0.51*
6	5.09	0.71*	6.31	0.80*
8	7.48	0.87*	7.29	0.86*
10	9.98	1.00*	10.82	1.03*
12	15.32	1.19*	16.52	1.22*
20	31.22	1.49	10.42	1.02

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.253$, $R^2 = 0.98$	$k = 0.249$, $R^2 = 0.95$

Time (hr)	Bacterial Concentration, cfu[a]/liter	
	Replicate 1	Replicate 2
0.5	1.29×10^7*[b]	4.30×10^7*
4	1.33*	4.20*
8.5	1.69* $(1.45 \pm 0.18) \times 10^7$	1.58* $(2.81 \pm 1.68) \times 10^7$
12	1.50*	1.14*

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters organism^{-1} hr^{-1}

Replicate 1	Replicate 2
$K = 1.75 \times 10^{-8}$	$K = 8.89 \times 10^{-9}$

Figure A-5. 100 ppb 2,4-DBE, Yakima River - Horn Rapids Park Boat Ramp (3-1-83)

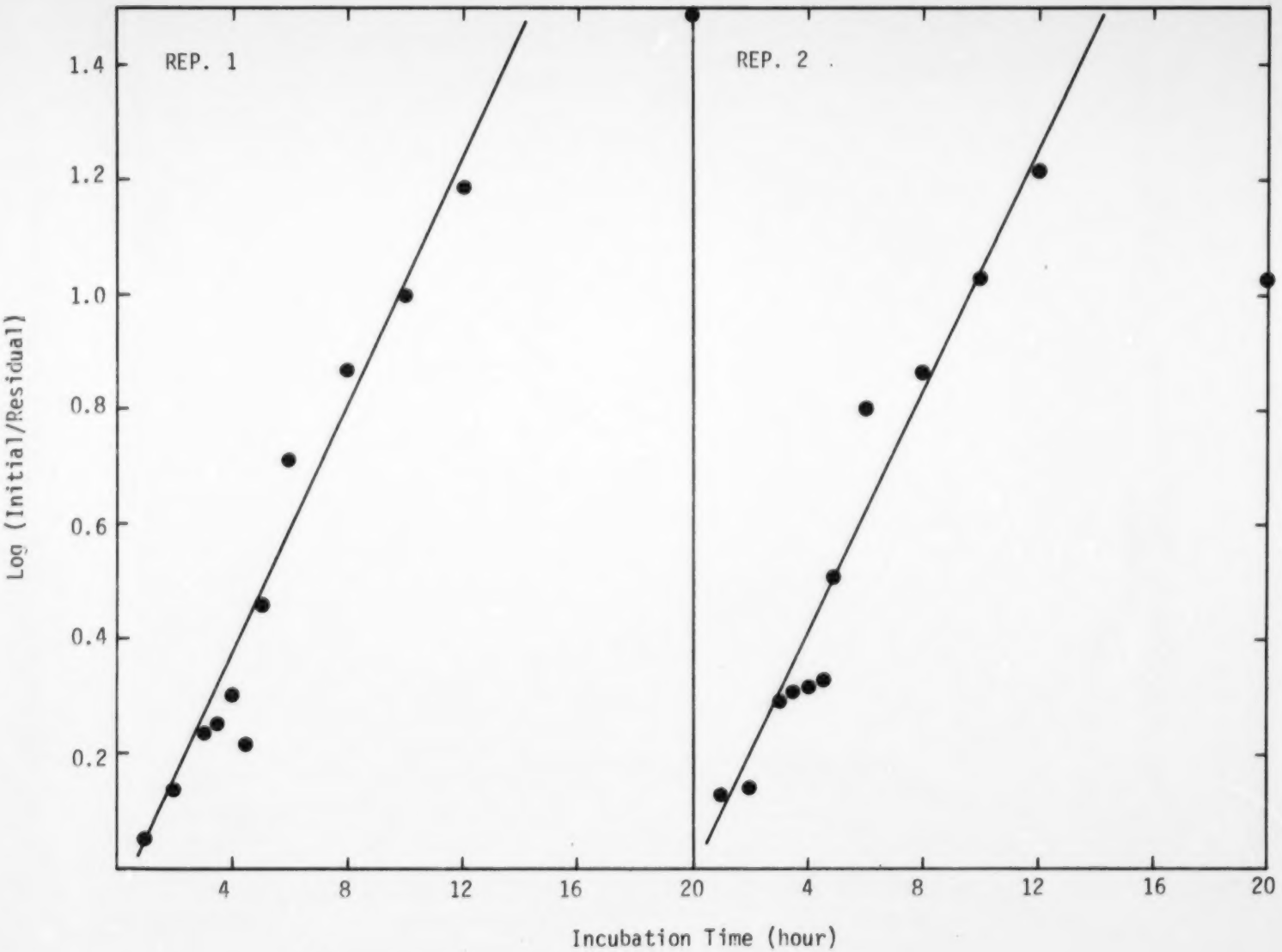


Table A-6. 100 ppb 2,4-DBE, Snake River - Hood Park Fish Pond (3-11-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
0	1.09	0.04*	1.12	0.049*
1	1.28	0.11*	0.937	-0.028*
2	1.44	0.16*	1.43	0.16*
3	1.36	0.14*	1.61	0.21*
4	3.57	0.55*	3.30	0.52*
5	3.38	0.53*	3.95	0.60*
6	4.03	0.61*	5.62	0.75*
8	11.30	1.05*	7.63	0.88*
9	25.48	1.41*	30.45	1.48*
10	29.09	1.46*	44.49	1.65*
12	55.49	1.77*	129.00	2.11*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.356$, $R^2 = 0.96$	$k = 0.407$, $R^2 = 0.95$

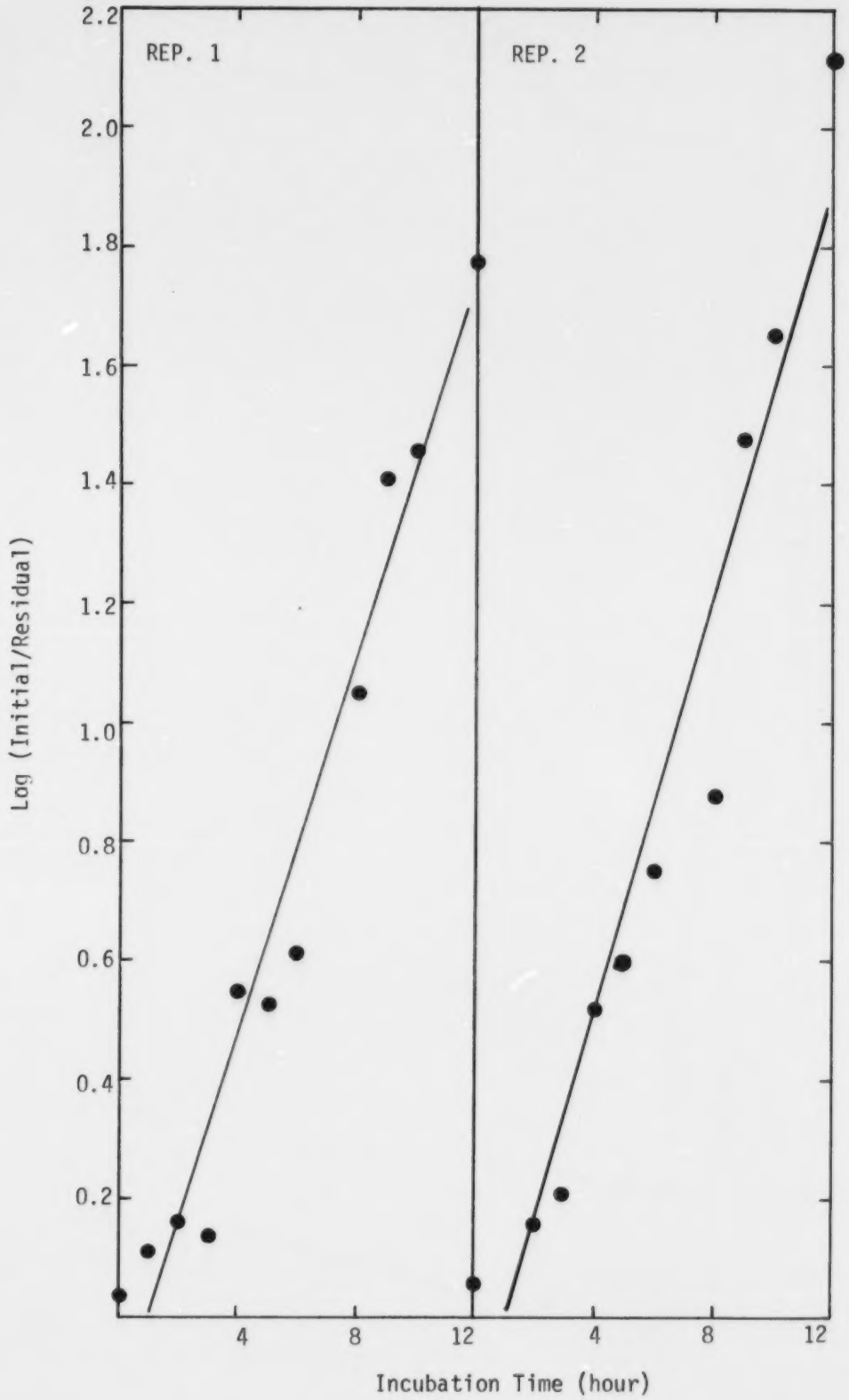
Time (hr)	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
2	0.38×10^6*[b]		0.38×10^6	
6	0.67*		0.70	
9	1.83*	$(1.42 \pm 1.11) \times 10^6$	1.58*	$(1.37 \pm 1.08) \times 10^6$
12	2.80*		2.80*	
16	3.03*		2.41*	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters organism^{-1} hr^{-1}

Replicate 1	Replicate 2
$K = 2.50 \times 10^{-7}$	$K = 2.97 \times 10^{-7}$

Figure A-6. 100 ppb 2,4-DBE, Snake River - Hood Park Fish Pond (3-11-83)



APPENDIX B

SECOND-ORDER RATE CONSTANTS FOR p-CRESOL

Table B-1. 1000 ppb p-Cresol, 331 Building Fish Pond (12-3-82)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
55	1.11	0.05	1.06	0.03
60	1.19	0.08	1.11	0.05
71	1.67	0.22*	1.37	0.14*
75	2.08	0.32*	1.66	0.22*
79	3.18	0.50*	2.02	0.31*
83	-	-	3.85	0.59*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.081$, $R^2 = 0.97$	$k = 0.083$, $R^2 = 0.90$

Time (hr)	Bacterial Concentration, cfu[a]/liter	
	Replicate 1	Replicate 2
30	2.43×10^7*[b]	3.37×10^7*
49	1.44* $(2.01 \pm 0.42) \times 10^7$	4.70* $(3.73 \pm 0.69) \times 10^7$
71	2.17*	3.73*
100	2.00*	3.13*

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 4.01 \times 10^{-9}$	$K = 2.22 \times 10^{-9}$

Figure B-1. 1000 ppb p-Cresol, 331 Building Fish Pond (12-3-82)

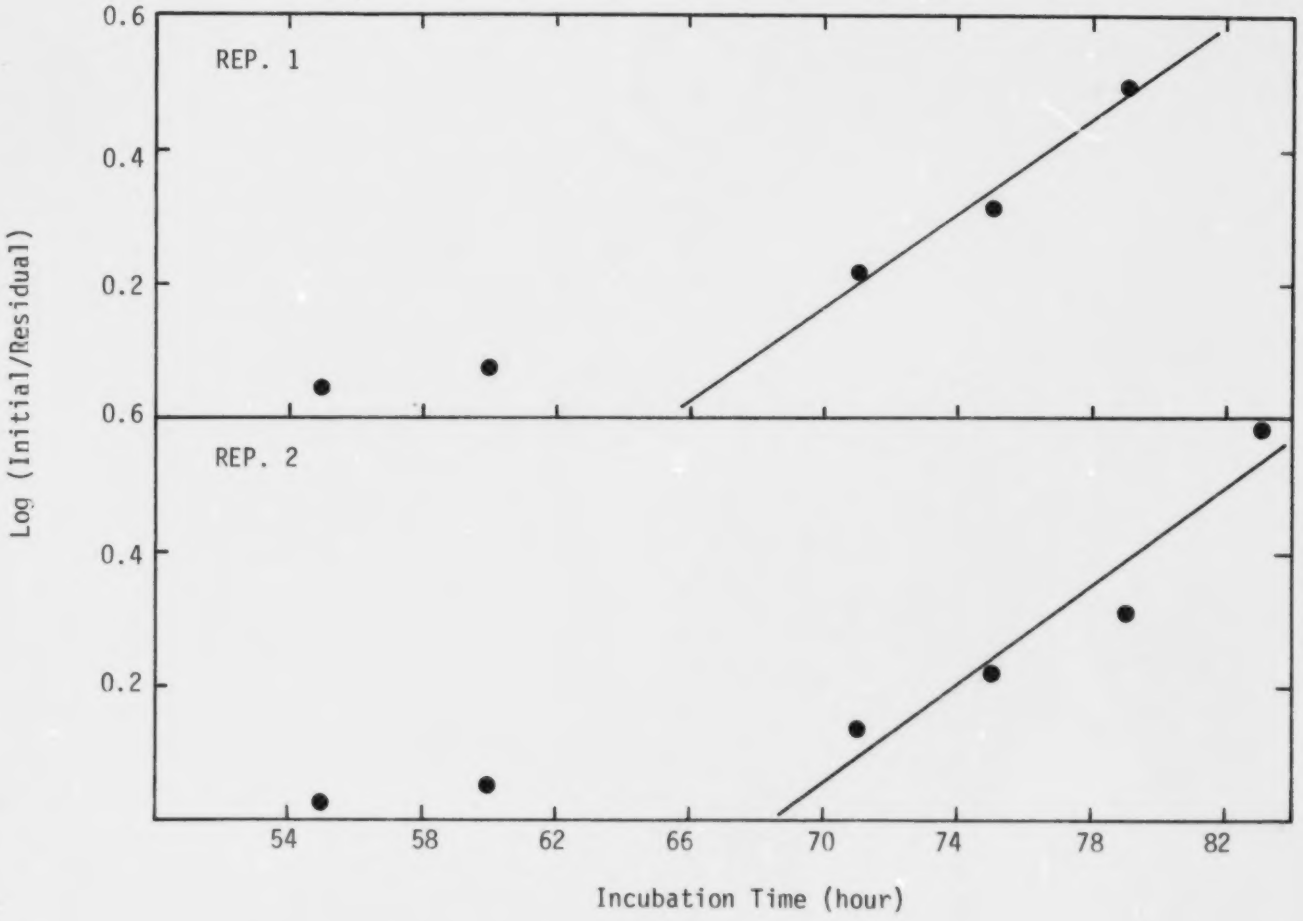


Table B-2. 100 ppb p-Cresol, 331 Building Fish Pond (12-3-82)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
22	-	-	1.10	0.04
26	1.11	0.05	1.13	0.05
30	1.11	0.04	1.28	0.11*
35	1.22	0.09	1.54	0.19*
49	1.89	0.28*	8.15	0.91*
55	2.86	0.46*	88.33	1.95
60	10.71	1.03*	-	-

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.154$, $R^2 = 0.89$	$k = 0.102$, $R^2 = 0.97$

Time (hr)	Bacterial Concentration, cfu[a]/liter	
	Replicate 1	Replicate 2
22	5.53×10^7*[b]	5.6×10^7*
30	5.57* $(5.51 \pm 0.38) \times 10^7$	3.83* $(4.28 \pm 1.16) \times 10^7$
49	5.00*	4.77*
71	5.93*	2.93*

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 2.80 \times 10^{-9}$	$K = 2.37 \times 10^{-9}$

Figure B-2. 100 ppb p-Cresol, 331 Building Fish Pond (12-3-82)

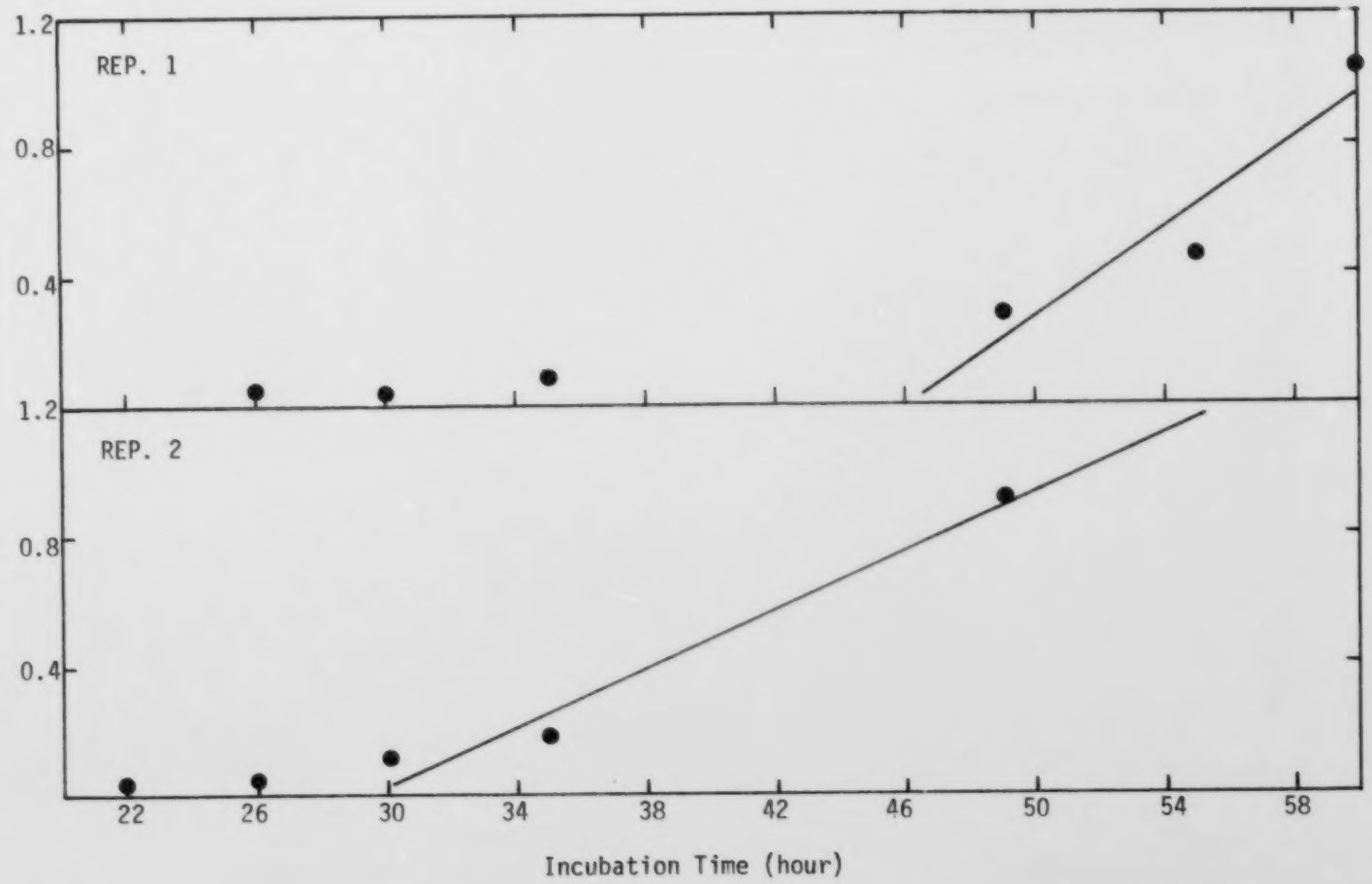


Table B-3. 1000 ppb p-Cresol, 331 Building Fish Pond (1-14-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
50	1.46	0.17	1.23	0.09
52	1.63	0.21	1.27	0.10
54	1.98	0.30	1.32	0.12
56	3.33	0.52*	1.47	0.17
58	7.93	0.90*	1.64	0.22*
60	18.89	1.28*	1.80	0.26*
62	64.60	1.81*	2.17	0.34*
64	88.24	1.95*	3.00	0.48*
66	-	-	3.21	0.51*
68	-	-	6.34	0.80*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.435$, $R^2 = 0.98$	$k = 0.124$, $R^2 = 0.91$

Time (hr)	Bacterial Concentration, cfu[a]/liter	
	Replicate 1	Replicate 2
50	1.03×10^7	0.59×10^7
54	10.3*	4.57*
60	51.7* $(40.77 \pm 26.73) \times 10^7$	5.67* $(5.74 \pm 1.22) \times 10^7$
66	60.3*	7.00*
74	16.2	1.72

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 1.06 \times 10^{-9}$	$K = 2.17 \times 10^{-9}$

Figure B-3. 1000 ppb p-Cresol, 331 Building Fish Pond (1-14-83)

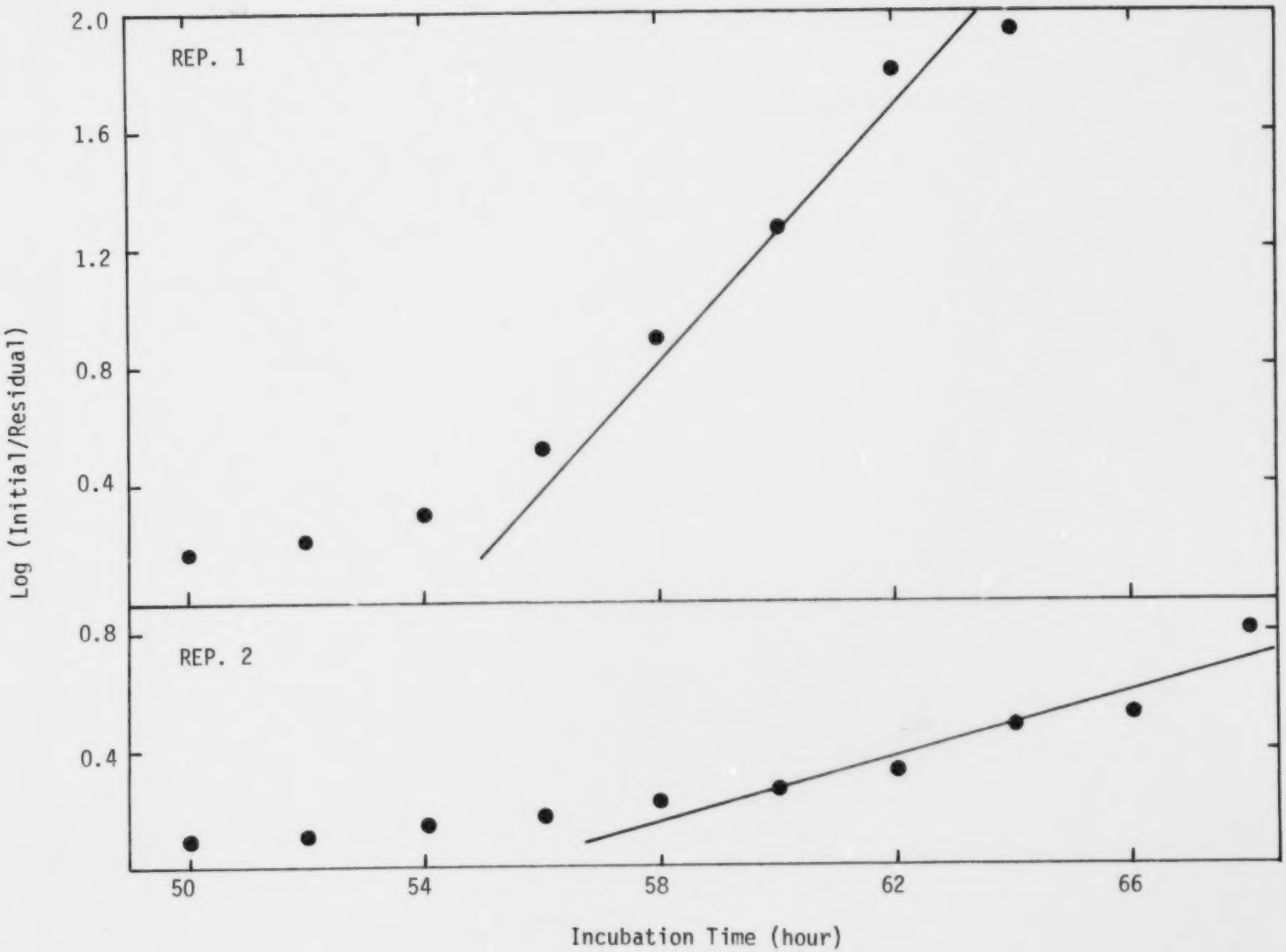


Table B-4. 100 ppb p-Cresol, 331 Building Fish Pond (1-14-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
26	1.38	0.14	1.46	0.16
28	1.47	0.17	1.47	0.17
30	1.65	0.22	1.48	0.17
32	1.68	0.22	1.77	0.25
34	2.17	0.34	2.24	0.35
36	2.61	0.42	2.73	0.44
38	3.41	0.53*	3.60	0.56*
40	5.16	0.71*	5.74	0.76*
42	12.29	1.09*	14.04	1.15*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.322$, $R^2 = 0.96$	$k = 0.340$, $R^2 = 0.97$

Time (hr)	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
26	1.12×10^7*[b]		0.71×10^7*	
30	4.10*		0.96*	
36	3.33*	$(4.70 \pm 1.75) \times 10^7$	2.00*	$(1.22 \pm 0.68) \times 10^7$
42	6.67*		-	
50	1.16		0.77	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 6.86 \times 10^{-9}$	$K = 2.78 \times 10^{-8}$

Figure B-4. 100 ppb p-Cresol, 331 Building Fish Pond (1-14-83)

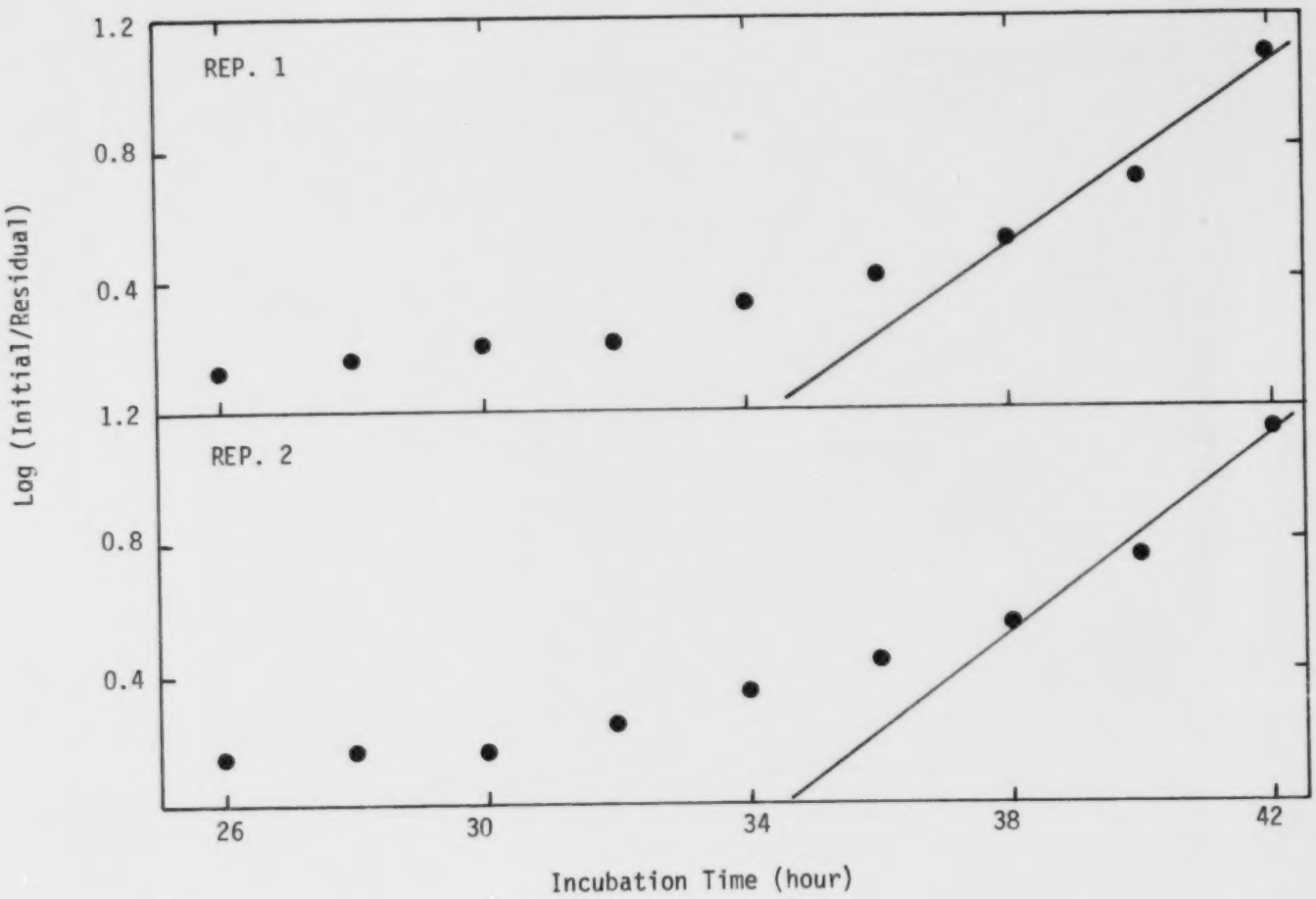


Table B-5. 1000 ppb p-Cresol, Yakima River - Horn Rapids Park Boat Ramp (2-7-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
27	1.10	0.04	1.08	0.03
28	1.09	0.04	1.10	0.04
29	1.12	0.05	1.14	0.06
30	1.14	0.06	1.14	0.06
31	1.14	0.06	1.11	0.05
33	1.23	0.09	1.25	0.10
35	1.24	0.09	1.26	0.10
37	1.30	0.11	1.32	0.12
39	1.35	0.13	1.40	0.15
41	1.41	0.15*	1.45	0.16*
48	2.15	0.33*	2.58	0.41*
52	3.13	0.50*	5.02	0.70*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.071$, $R^2 = 0.98$	$k = 0.110$, $R^2 = 0.96$

Time (hr)	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
26	0.86×10^7		1.58×10^7	
30.5	0.64		1.65	
35	0.93	$(0.82 \pm 0.23) \times 10^7$	1.81	$(4.99 \pm 3.43) \times 10^7$
41	0.63*[b]		1.26*	
50	1.08*		8.00*	
55	0.76*		5.70*	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 8.73 \times 10^{-9}$	$K = 2.20 \times 10^{-9}$

Figure B-5. 1000 ppb p-Cresol, Yakima River - Horn Rapids Park Boat Ramp (2-7-83)

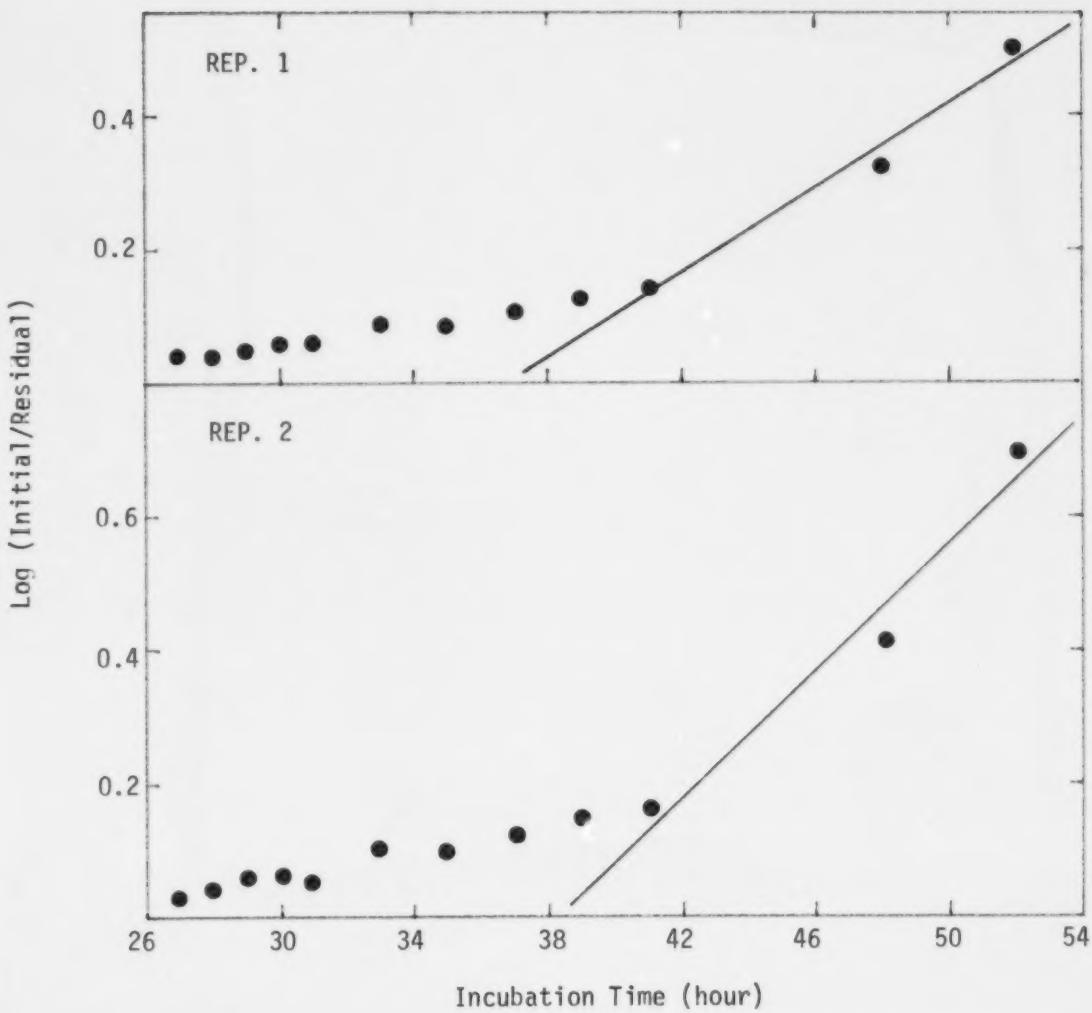


Table B-6. 100 ppb p-Cresol, Yakima River - Horn Rapids Park Boat Ramp (2-7-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
1	1.06	0.02*	1.15	0.06*
2	1.09	0.04*	1.22	0.08*
3	1.06	0.02*	1.21	0.08*
5	1.13	0.05*	1.23	0.09*
6	1.17	0.07*	1.31	0.12*
7	1.27	0.10*	1.43	0.15*
9	1.28	0.11*	1.48	0.17*
11	1.43	0.15*	1.63	0.21*
13	1.56	0.19*	1.84	0.27*
15	1.85	0.27*	2.17	0.34*
17	2.06	0.31*	2.78	0.44*
24	15.91	1.20	36.37	1.56

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.041$, $R^2 = 0.95$	$k = 0.051$, $R^2 = 0.93$

Time (hr)	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
2	1.15×10^7*[b]		1.69×10^7*	
6.5	1.04*		0.70*	
11	1.44*	$(1.24 \pm 0.19) \times 10^7$	1.27*	$(1.19 \pm 0.37) \times 10^7$
17	1.14*		1.28*	
26	1.44*		1.01*	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 3.34 \times 10^{-9}$	$K = 4.25 \times 10^{-9}$

Figure B-6. 100 ppb p-Cresol, Yakima River - Horn Rapids Park Boat Ramp (2-7-83)

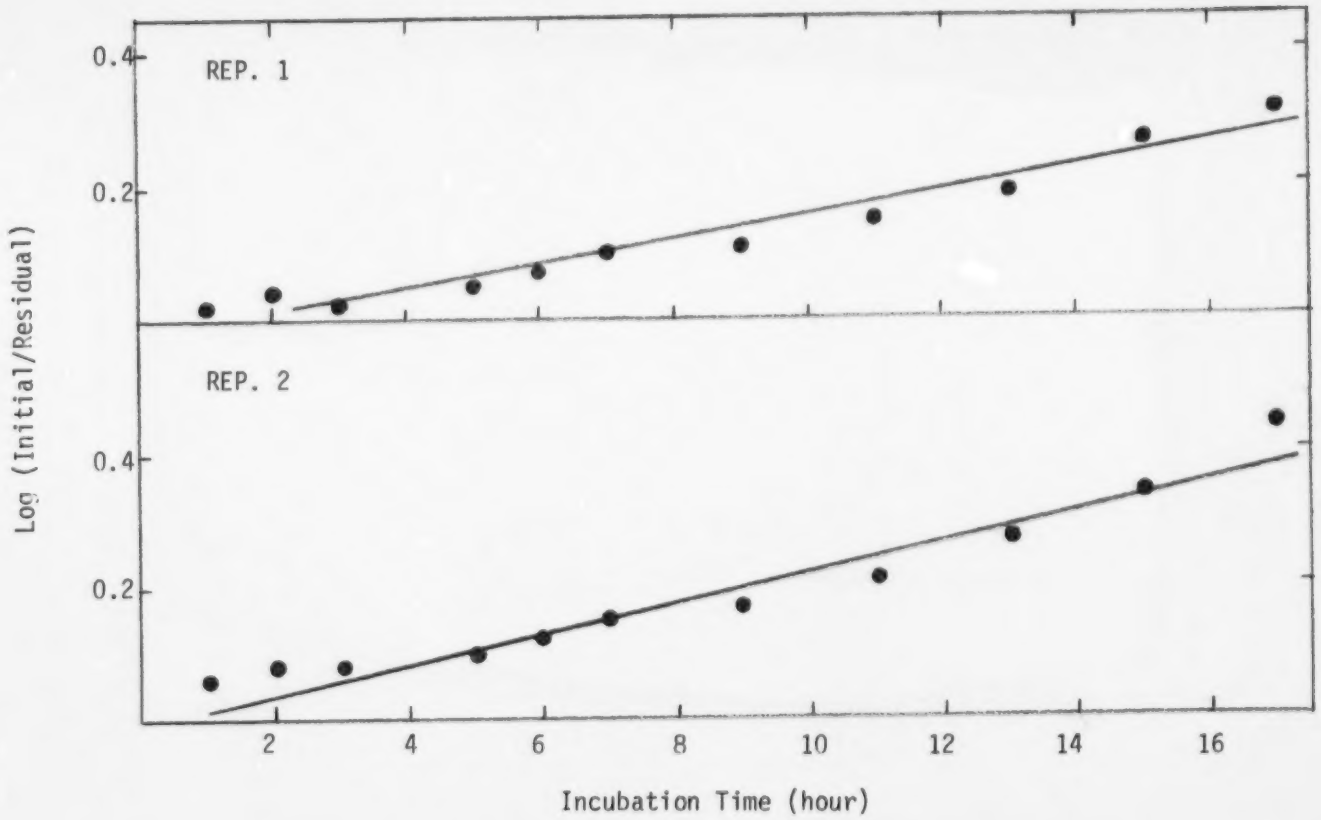


Table B-7. 1000 ppb p-Cresol, Columbia River - Richland "Wye" Marina (2-14-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
52	1.37	0.14*	1.28	0.11
53	1.71	0.24*	1.73	0.24
54	1.93	0.29*	1.74	0.24
55	2.37	0.37*	2.09	0.32
56	3.05	0.48*	2.21	0.35
57	28.97	1.46	2.71	0.43
59	8.88	0.95*	3.67	0.56*
61	17.32	1.24*	16.48	1.22*
63	38.19	1.58*	85.92	1.93*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.305$, $R^2 = 0.98$	$k = 0.790$, $R^2 = 1.00$

Time (hr)	Bacterial Concentration, cfu[a]/liter	
	Replicate 1	Replicate 2
50	1.25×10^7*[b]	12.57×10^7*
54.5	8.13 $(1.63 \pm 0.89) \times 10^7$	14.77* $(13.67 \pm 1.56) \times 10^7$
59.0	2.64*	2.61
65.5	0.99*	730

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters organism^{-1} hr^{-1}

Replicate 1	Replicate 2
$K = 1.87 \times 10^{-8}$	$K = 5.76 \times 10^{-9}$

Figure B-7. 1000 ppb p-Cresol, Columbia River - Richland "Wye" Marina (2-14-83)

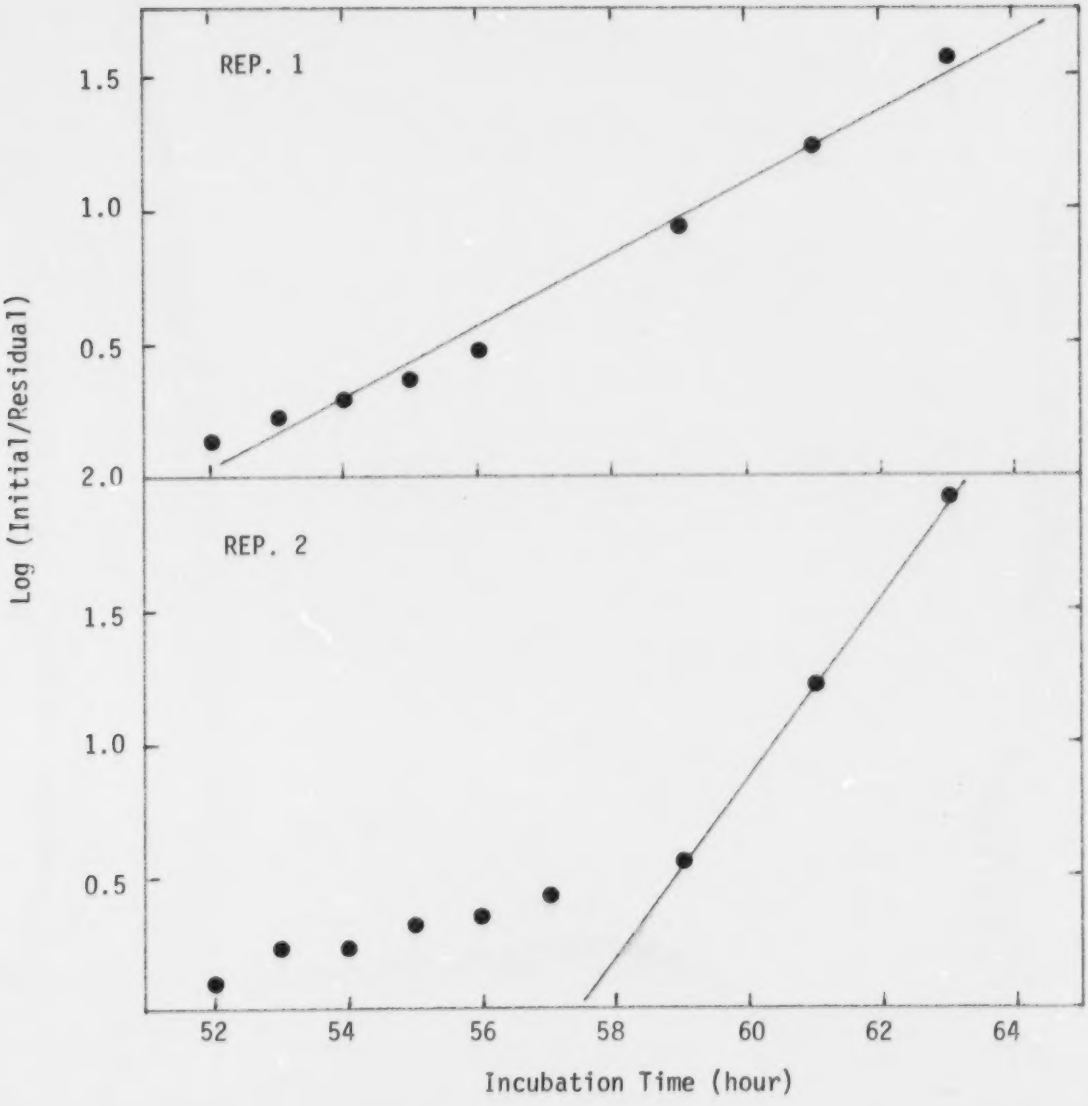


Table B-8. 1000 ppb p-Cresol, Walla Walla River - Madame Dorion Park (3-28-83)

Incubation Time (hr)	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
48	1.47	0.17*	1.49	0.17*
49	1.48	0.17*	1.54	0.19*
50	1.61	0.21*	1.71	0.23*
51	1.64	0.21*	1.76	0.25*
52	1.76	0.24*	1.89	0.28*
53	1.99	0.30*	2.16	0.33*
54	2.00	0.30*	2.19	0.34*
55	2.28	0.36*	2.63	0.42*
56	2.48	0.39*	3.03	0.48*
58	3.58	0.55*	4.79	0.68*

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

Replicate 1	Replicate 2
$k = 0.082$, $R^2 = 0.92$	$k = 0.107$, $R^2 = 0.92$

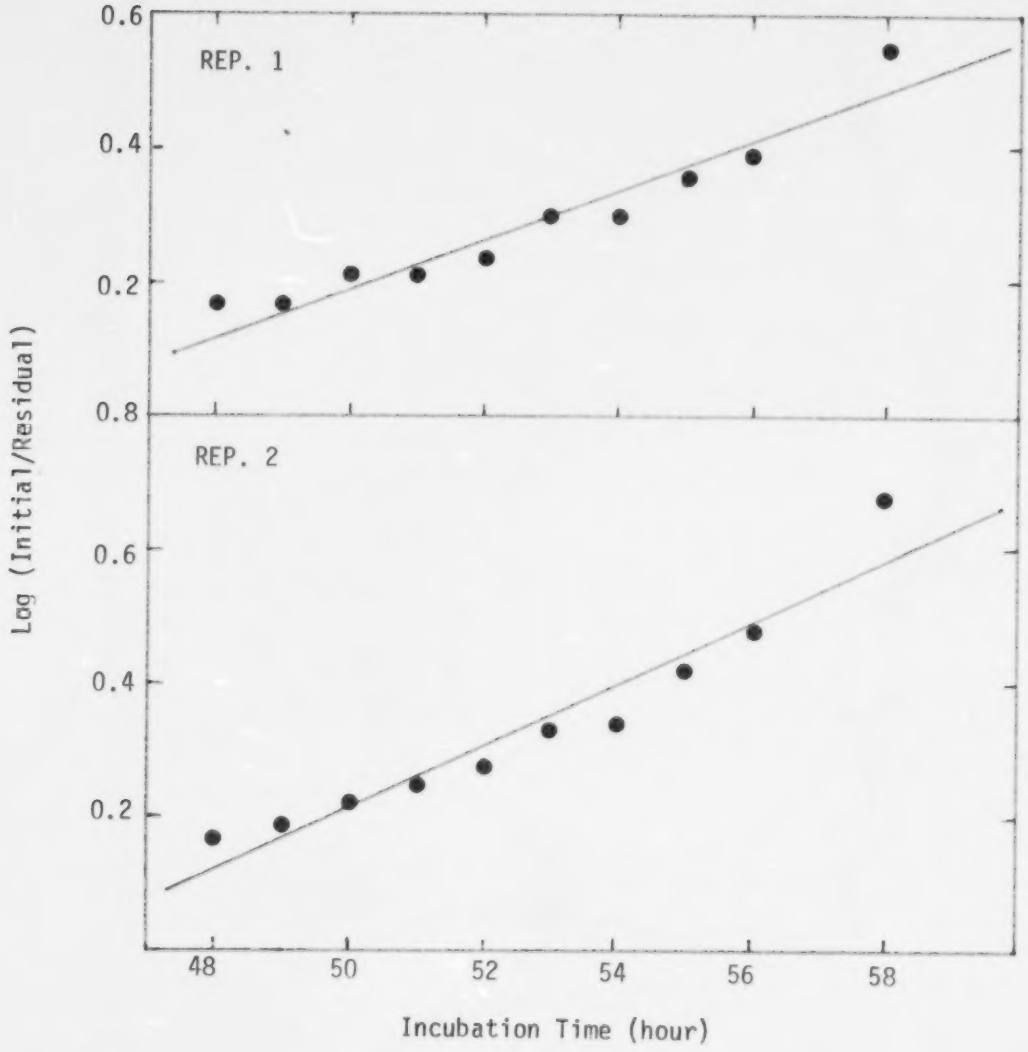
Time (hr)	Bacterial Concentration, cfu[a]/liter	
	Replicate 1	Replicate 2
32	3.83×10^{6}*[b]	4.73×10^{6}*
50.5	3.13* $(3.52 \pm 0.36) \times 10^{6}$	3.90* $(4.12 \pm 0.54) \times 10^{6}$
54.5	3.60*	3.73*
60	25.6	18.73

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

Replicate 1	Replicate 2
$K = 2.32 \times 10^{-8}$	$K = 2.59 \times 10^{-8}$

Figure B-8. 1000 ppb p-Cresol, Walla Walla River - Madame Dorion Park (3-28-83)



APPENDIX C

SECOND-ORDER RATE CONSTANTS FOR QUINOLINE

Table C-1. Replicate Amendment Study, 1 ppm Quinoline, Yakima River - Horn Rapids Park Boat Ramp (5-2-83)

Incubation Time (days)		Replicate 1 Tnit./Res.	Replicate 1 Log		Replicate 2 Tnit./Res.	Replicate 2 Log
0		sl[a]	sl		sl	sl
1		sl	sl		sl	sl
2		1.08	0.03		1.14	0.06
3		0.91	-0.04		1.04	0.02
4		0.96	-0.02		1.22	0.09
5		1.02	0.01		1.19	0.08
6		1.18	0.07		1.01	0.00
7		nd[b]	nd		1.57	0.20
8		nd	nd		nd	nd
9	2nd Amend. →	0.81	-0.09		nd	nd
10		nd	nd		nd	nd
11		nd	nd	2nd Amend. →	0.88	-0.05
12		nd	nd		nd	nd
13		nd	nd		nd	nd
14		nd	nd		0.96	-0.02
15	3rd Amend. →	0.97	-0.01	3rd Amend. →	nd	nd
16		2.47	0.39		nd	nd
17		nd	nd		nd	nd
18		nd	nd		nd	nd
19		nd	nd		nd	nd
20		nd	nd		nd	nd
21		nd	nd		nd	nd

[a] sl = sample lost

[b] nd = not detectable

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	DNS	DNS
3rd Amend.	DNS	DNS

DNS = data not sufficient to determine pseudo first-order rate constants

Table C-1. (Continued)

Incubation Time (days)		Bacterial Concentration, cfu[a]/liter Replicate 1		Replicate 2
0		1.14×10^6		2.33×10^6
1		2.88		3.57
2		3.77		5.17
3		5.23		9.07
5		1.10		3.43
6		1.95		2.01
7		TNTC[b]		2.70
8		6.80		2.22
9	2nd Amend. →	12.83	2nd Amend. →	4.17
12		0.80		4.80
13		1.20		4.00
14		2.45		6.40
15	3rd Amend. →	4.60	3rd Amend. →	5.00
16		1.11		1.60
19		1.89		0.30
20		0.63		0.83

[a]cfu = colony forming units, average of three plates

[b]TNTC = too numerous to count

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	DNS	DNS
3rd Amend.	DNS	DNS

DNS = data not sufficient to determine second-order rate constants

Figure C-1. Replicate Amendment Study, 1 ppm Quinoline, Yakima River - Horn Rapids Park Boat Ramp (5-2-83)

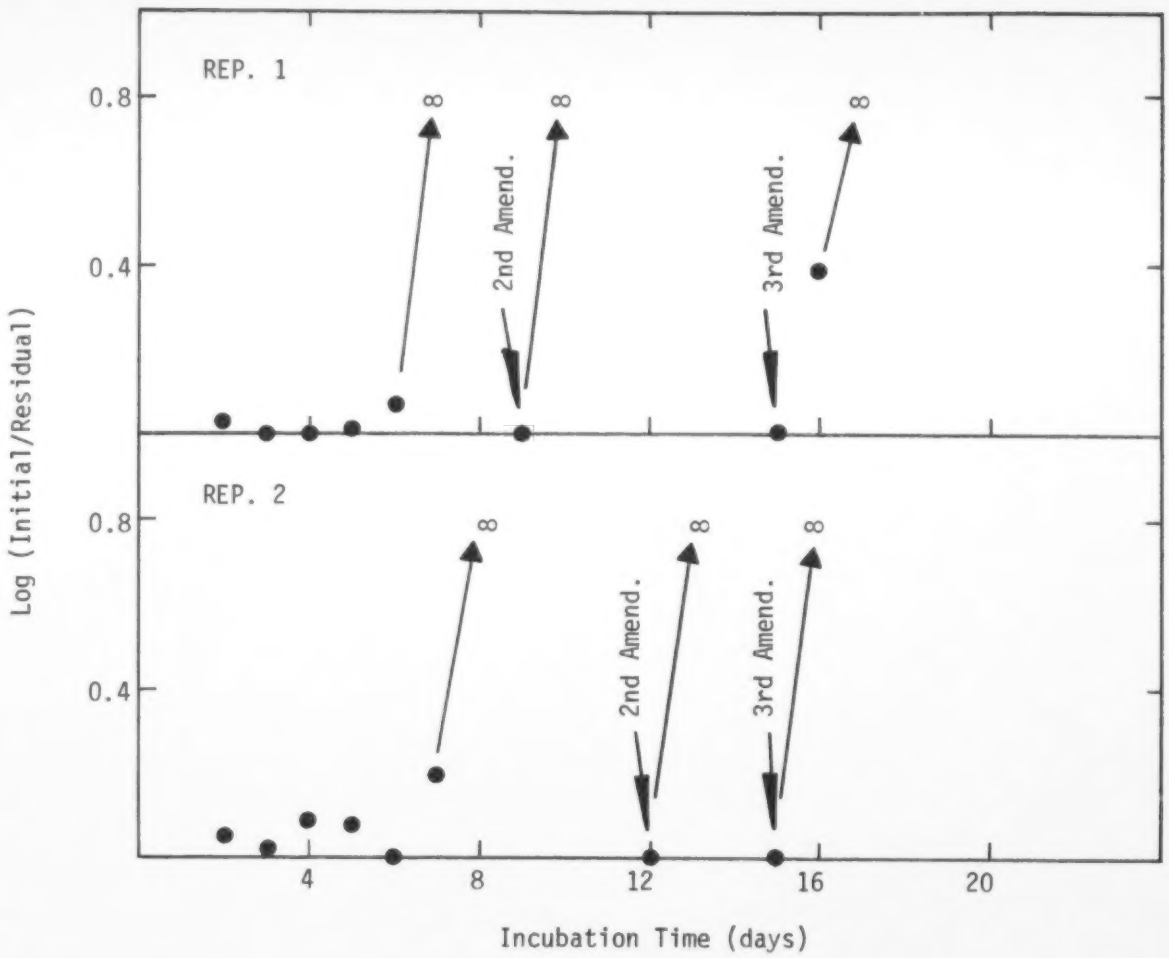


Table C-2. Replicate Amendment Study, 1.0 ppm Quinoline, Snake River - Hood Park Fish Pond (5-2-83)

Incubation Time (days)		Replicate 1 Init./Res.	Replicate 1 Log		Replicate 2 Init./Res.	Replicate 2 Log
0		1.02	0.01		1.07	0.03
1		1.00	0.00		1.12	0.05
2		1.21	0.08		0.97	-0.01
3		1.00	0.00		1.04	0.02
4		0.96	-0.02		1.07	0.03
5		0.98	-0.01		1.08	0.03
6		0.98	-0.01		1.06	0.02
7		1.13	0.05		1.11	0.04
8		0.96	-0.02		1.01	0.00
9		0.94	-0.03		1.27	0.10*[c]
10		1.48	0.17		2.57	0.41*
11		nd[a]	nd		5.67	0.75*
12		nd	nd		nd	nd
13	2nd Amend. →	0.95	-0.02	2nd Amend. →	0.95	-0.01
14		2.68	0.43		1.53	0.18
15		nd	nd		4.55	0.66
16		nd	nd		nd	nd
17		nd	nd		nd	nd
18		nd	nd		nd	nd
19		nd	nd		nd	nd
20	3rd Amend. →	sl[b]	sl	3rd Amend. →	sl	sl
21		sl	sl		sl	sl
22		nd	nd		14.99	1.18
23		nd	nd		nd	nd

[a] nd = not detectable

[b] sl = sample lost

[c] values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	$k = 3.12 \times 10^{-2}$, $R^2 = 1.00$
2nd Amend.	DNS	DNS
3rd Amend.	DNS	DNS

DNS = data not sufficient to determine pseudo first-order rate constants

Table C-2. (Continued)

Incubation Time (days)	Bacterial Concentration, cfu[a]/liter				
	Replicate 1		Replicate 2		
0		0.45×10^6		0.38×10^6	
1		1.38		1.63	
2		1.44		1.22	
3		1.28		1.41	
5		0.36		0.61	
6		0.47		0.52	
7		0.59		2.02	
8		1.03		1.38	
9		0.65		38.57*[b]	33.69 ± 6.91
12		3.13		28.80*	
13	2nd Amend. →	29.20	2nd Amend. →	31.40	
14		11.23		41.40	
15		4.60		5.00	
16		6.97		47.53	
19		5.20		103.33	
20	3rd Amend. →	18.93	3rd Amend. →	134.00	
21		6.07		187.33	
22		34.33		111.00	
23		16.00		68.33	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters organism^{-1} hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	$K = 9.26 \times 10^{-10}$
2nd Amend.	DNS	DNS
3rd Amend.	DNS	DNS

DNS = data not sufficient to determine second-order rate constants

Figure C-2. Replicate Amendment Study, 1.0 ppm Quinoline, Snake River - Hood Park Fish Pond (5-2-83)

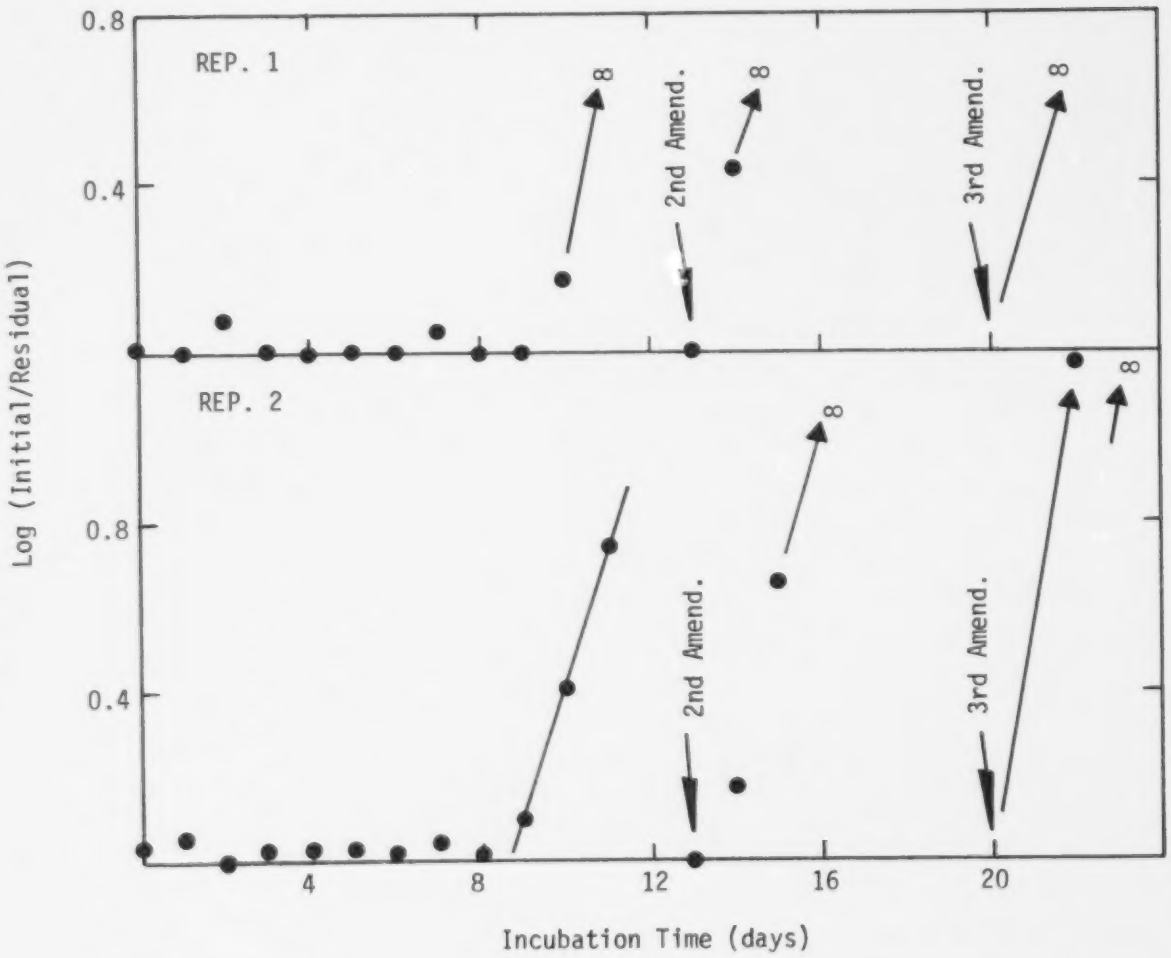


Table C-3. Replicate Amendment Study, 1.0 ppm Quinoline, Walla Walla River - Madame Dorion Park (5-2-83)

Incubation Time (days)		Replicate 1 Init./Res.	Replicate 1 Log		Replicate 2 Init./Res.	Replicate 2 Log
0		1.05	0.02		1.12	0.05
1		0.98	-0.01		1.12	0.05
2		1.08	0.03		1.08	0.03
3		1.00	0.00		0.95	-0.02
4		0.95	-0.02		1.11	0.04
5		1.95	0.29		1.37	0.14
6		nd[a]	nd		nd	nd
7		nd	nd		nd	nd
8		nd	nd		nd	nd
9	2nd Amend. →	0.83	-0.08	2nd Amend. →	0.88	-0.06
10		10.04	1.00		nd	nd
11		nd	nd		nd	nd
12		nd	nd		nd	nd
13		nd	nd		nd	nd
14		nd	nd		nd	nd
15	3rd Amend. →	0.99	-0.01	3rd Amend. →	0.99	0.00
16		1.81	0.26*[b]		1.44	0.16*
17		2.17	0.34*		3.26	0.51*
18		2.73	0.44*		3.90	0.59*
19		3.62	0.56*		4.44	0.65*
20		5.06	0.70*		5.63	0.75*
21		7.11	0.85*		6.39	0.81*
22		9.61	0.98*		7.86	0.90*
23		11.23	1.05*		6.87	0.84*

[a] nd = not detectable

[b] values with * were used to determine pseudo first-order rate constants in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	DNS	DNS
3rd Amend.	$k = 1.15 \times 10^{-2}$, $R^2 = 0.99$	$k = 8.53 \times 10^{-3}$, $R^2 = 0.84$

DNS = data not sufficient to determine pseudo first-order rate constants

Table C-3. (Continued)

Incubation Time (days)	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
0	3.43×10^6		4.60×10^6	
1	5.43		6.63	
2	9.33		6.17	
3	6.30		6.90	
5	4.60		4.87	
6	15.00		TNTC[c]	
7	1.51		1.54	
8	4.07		18.13	
9	2nd Amend. → 15.37		2nd Amend. → 32.63	
12	2.33		5.20	
13	0.69		4.53	
14	3.27		3.10	
15	3rd Amend. → 7.73		3rd Amend. → 8.60	
16	4.43*[b]	2.64 ± 1.76	1.64*	2.55 ± 1.97
19	1.19*		1.12*	
20	1.62*		6.10*	
21	0.55*		0.74*	
22	4.73*		3.30*	
23	3.30*		2.41*	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants in liters organism^{-1} hr^{-1}

[c]TNTC = too numerous to count

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	DNS	DNS
3rd Amend.	$K = 4.37 \times 10^{-9}$	$K = 3.35 \times 10^{-9}$

DNS = data not sufficient to determine second-order rate constants

Figure C-3. Replicate Amendment Study, 1.0 ppm Quinoline, Walla Walla River - Madame Dorion Park (5-2-83)

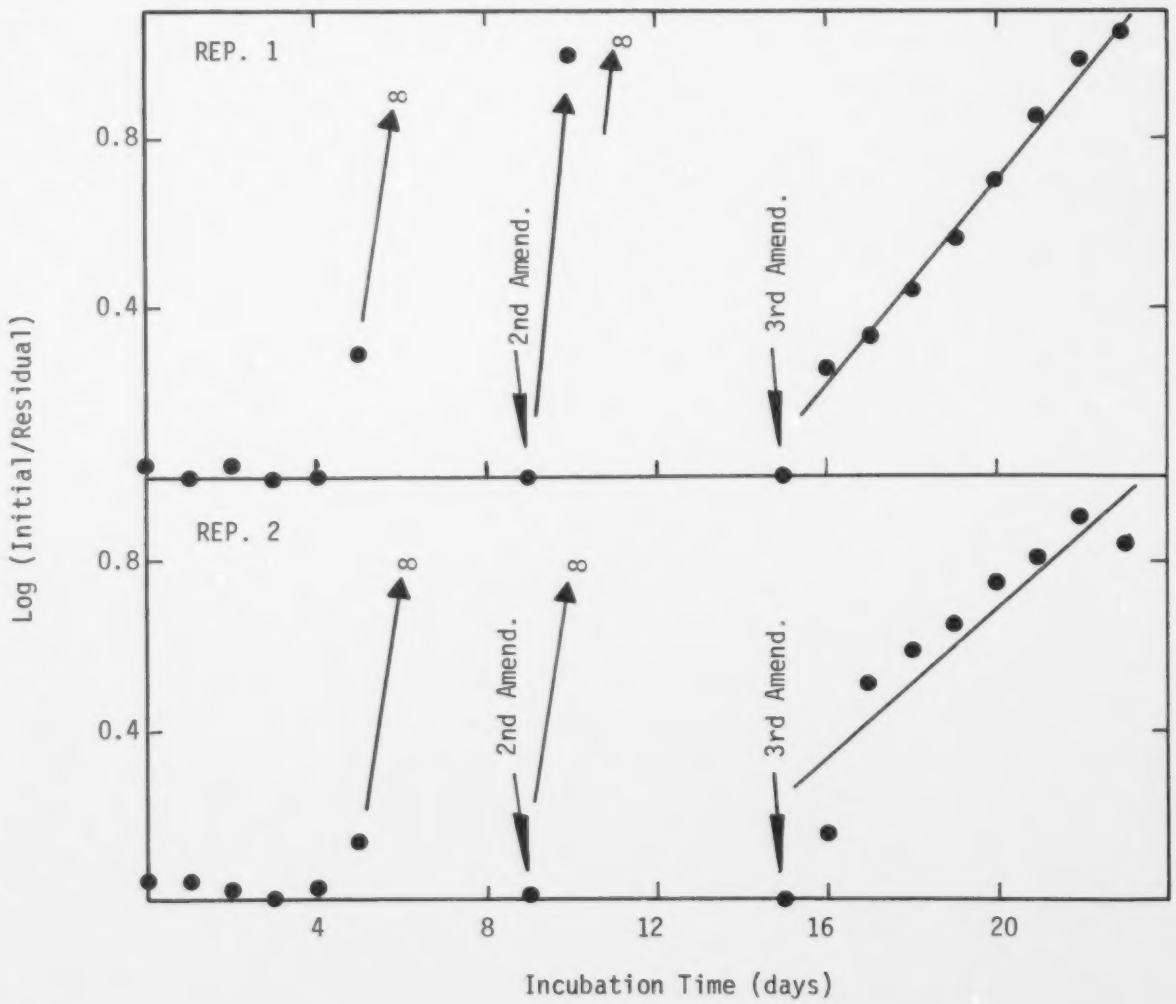


Figure C-4. Replicate Amendment Study, 1.0 ppm Quinoline, Snake River - Hood Park Boat Ramp (8-1-83)

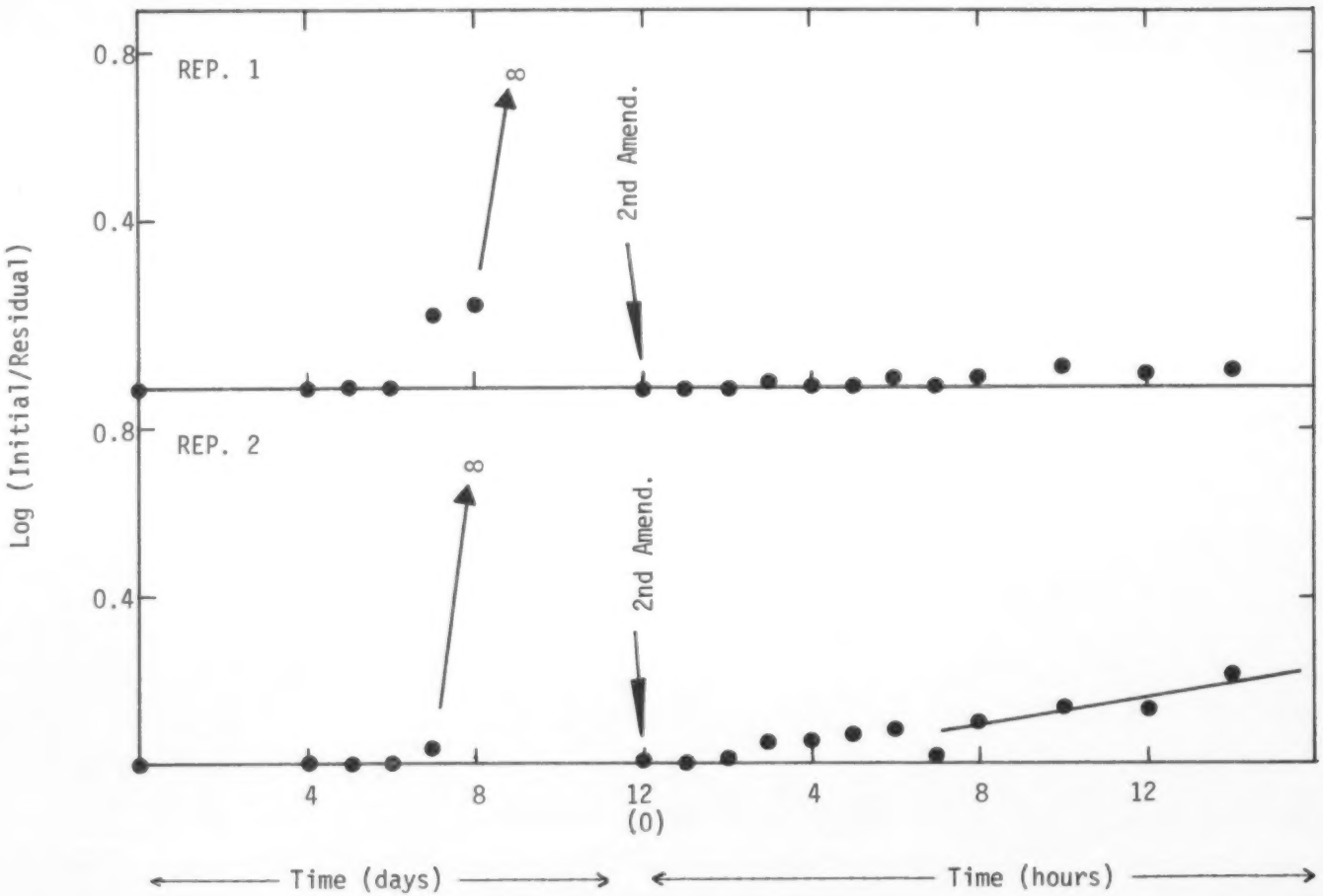


Table C-4. Replicate Amendment Study, 1.0 ppm Quinoline, Snake River - Hood Park Boat Ramp (8-1-83)

Incubation Time	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
1st Amend. (days)				
0	1.01	0.00	0.93	-0.03
4	0.79	-0.10	0.81	-0.09
5	0.98	-0.01	0.98	-0.01
6	0.99	-0.01	0.98	-0.01
7	1.51	0.18	1.11	0.04
8	1.59	0.20	nd	nd
9	nd[a]	nd		
		2nd Amendment on day 12		
2nd Amend. (hours)				
0	0.90	-0.05	0.87	-0.06
1	0.97	-0.01	1.01	0.00
2	0.98	-0.01	1.06	0.02
3	1.02	0.01	1.13	0.05
4	1.01	0.00	1.15	0.06
5	1.00	0.00	1.18	0.07
6	1.05	0.02	1.20	0.08
7	0.89	-0.05	1.02	0.01
8	1.04	0.02	1.26	0.10*[b]
10	1.11	0.05	1.37	0.14*
12	1.06	0.03	1.34	0.13*
14	1.10	0.04	1.62	0.21*

[a]nd = not detectable

[b]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	DNS	$k = 0.037$, $R^2 = 0.79$

DNS = data not sufficient to determine pseudo first-order rate constants

Time	Bacterial Concentration, cfu[a]/liter		
	Replicate 1	Replicate 2	
1st Amend. (days)			
0	1.97×10^7	1.88×10^7	
2nd Amend. (hours)			
3	2.33	35.30	
6.5	2.49	22.60*[b]	25.47 ± 2.51
10	1.81	27.30*	
14	2.26	26.50*	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	DNS	$K = 1.45 \times 10^{-10}$

DNS = data not sufficient to determine second-order rate constants

Table C-5. Replicate Amendment Study, 1.0 ppm Quinoline, Columbia River - Richland "Wye" Marina (8-1-83)

Incubation Time	Replicate 1 Init./Res.	Replicate 1 Log	Replicate 2 Init./Res.	Replicate 2 Log
1st Amend. (days)				
0	1.01	0.00	1.02	0.01
4	0.92	-0.03	0.97	-0.02
5	0.85	-0.07	0.91	-0.04
6	0.89	-0.05	1.00	0.00
7	0.92	-0.03	1.15	0.06
8	1.13	0.05	98.0	1.99
9	12.43	1.09	nd[b]	nd
		2nd Amendment on day 12		
2nd Amend. (hours)				
0	0.96	-0.02	1.00	0.00
1	0.98	-0.01	1.13	0.05
2	0.96	-0.02	0.96	-0.02
3	1.08	0.03	1.09	0.04
4	1.30	0.11	1.25	0.10
5	1.24	0.09	1.19	0.08
6	1.24	0.09*[a]	1.25	0.10
7	1.31	0.12*	1.28	0.11
8	1.40	0.15*	1.36	0.13
10	1.42	0.15*	1.47	0.17*
12	1.97	0.30*	2.26	0.36*
14	2.18	0.34*	4.00	0.60*

[a] values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

[b] nd = not detectable

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$k = 0.073$, $R^2 = 0.92$	$k = 0.248$, $R^2 = 1.00$

DNS = data not sufficient to determine pseudo first-order rate constants

Time	Bacterial Concentration, cfu[a]/liter Replicate 1		Replicate 2	
1st Amend. (days)				
0	0.76×10^7		1.13×10^7	
2nd Amend. (hours)				
3	13.90		4.00	
6.5	16.10*[b]		4.60	
10	17.60*	18.27 ± 2.57	4.13*	4.90 ± 1.09
14	21.10*		5.67*	

[a] cfu = colony forming units, average of three plates

[b] values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$K = 4.02 \times 10^{-10}$	$K = 5.05 \times 10^{-9}$

DNS = data not sufficient to determine second-order rate constants

Figure C-5. Replicate Amendment Study, 1.0 ppm Quinoline, Columbia River - Richland "Wye" Marina (8-1-83)

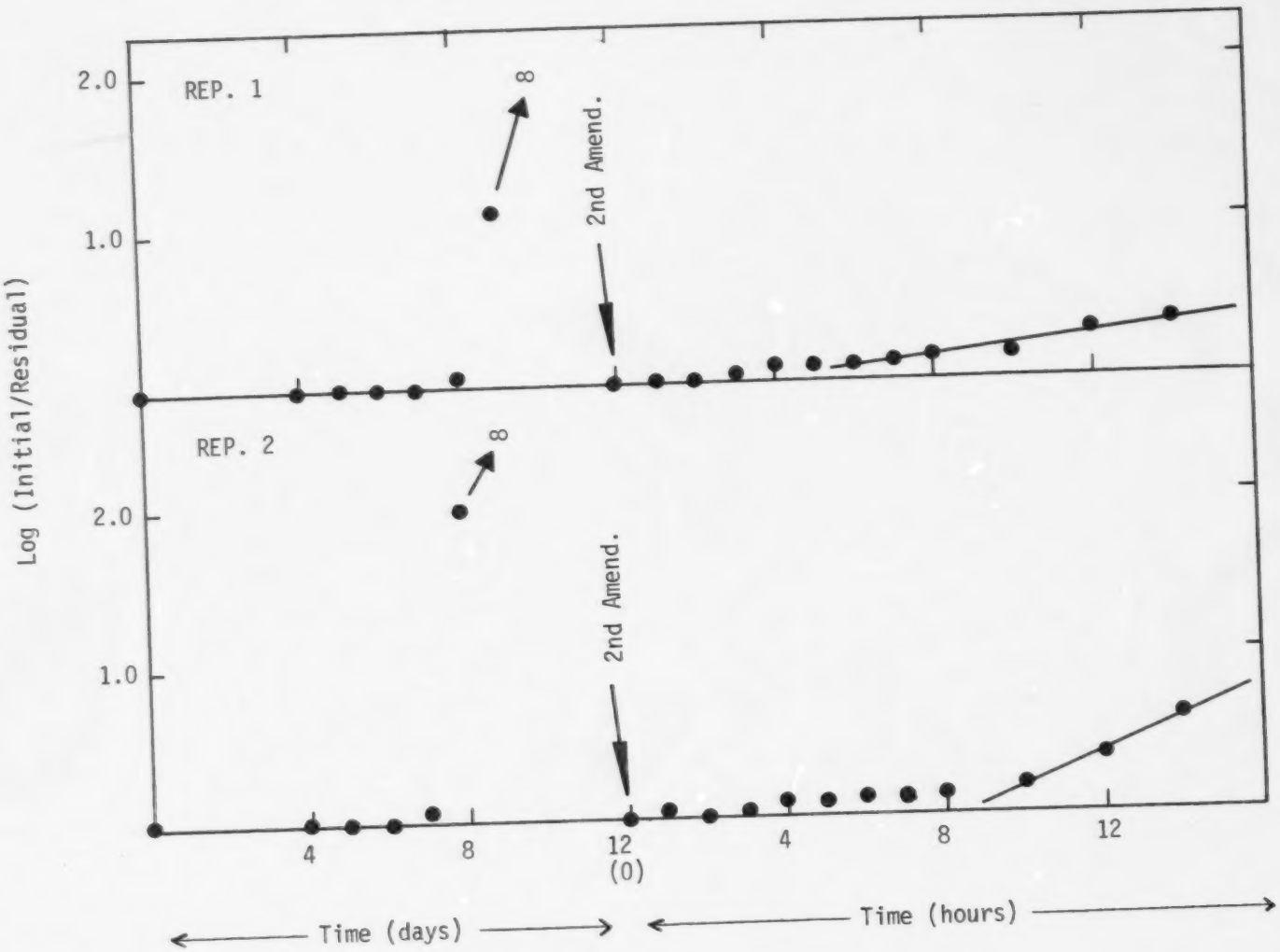


Table C-6. Replicate Amendment Study, 1.0 ppm Quinoline, Yakima River - Horn Rapids Park Boat Ramp (8-1-83)

Incubation Time	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
1st Amend. (days)				
0	0.97	-0.01	0.89	-0.005
4	4.78	0.68	2.00	0.30
5	nd[a]	nd	nd	nd
6				
	2nd Amendment on day 7			
2nd Amend. (hours)				
0	1.05	0.02	1.10	0.04
1	1.00	0.00	1.06	0.02
2	1.00	0.00	1.06	0.02
3	0.99	-0.01	1.02	0.01
5	1.10	0.04	1.27	0.10
6	1.15	0.06	1.42	0.15
7	1.20	0.08	1.56	0.19
8	1.35	0.13	1.91	0.28*
10	1.36	0.13*[b]	2.93	0.47*
12	2.26	0.35*	18.50	1.27*
14	23.00	1.36*	nd	nd
26	nd	nd		

[a] nd = not detectable

[b] values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	$k = 0.570$, $R^2 = 0.89$
2nd Amend.	$k = 0.708$, $R^2 = 0.88$	

DNS = data not sufficient to determine pseudo first-order rate constants

Time	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
1st Amend. (days)				
0	1.00×10^7		1.10×10^7	
2nd Amend. (hours)				
0.5	0.18		0.24	
4.5	0.16		0.19	
10	0.19*[b]	0.22 ± 0.04	0.18*	0.15 ± 0.05
14	0.24*		0.11*	

[a] cfu = colony forming units, average of three plates

[b] values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	$K = 3.80 \times 10^{-7}$
2nd Amend.	$K = 3.22 \times 10^{-7}$	

DNS = data not sufficient to determine second-order rate constants

Figure C-6. Replicate Amendment Study, 1.0 ppm Quinoline, Yakima River - Horn Rapids Park Boat Ramp (8-1-83)

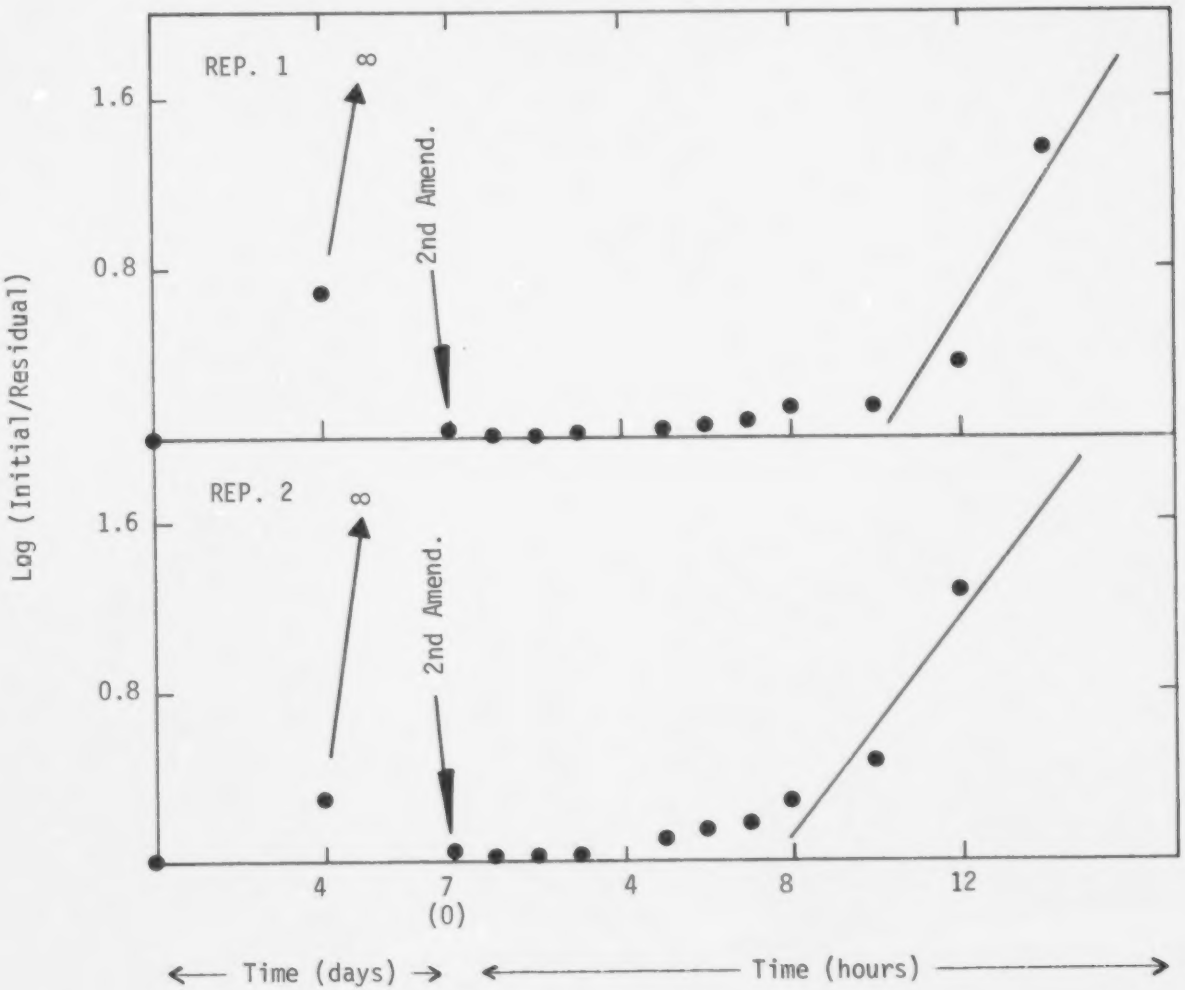


Table C-7. Replicate Amendment Study, 1.0 ppm Quinoline, Walla Walla River - Madame Dorion Park (9-7-83)

Incubation Time	Replicate 1 Init./Res.	Replicate 1 Log	Replicate 2 Init./Res.	Replicate 2 Log
1st Amend. (days)				
0	0.97	-0.01	1.05	0.02
3	0.95	-0.02	0.94	-0.03
4	1.01	-0.003	0.97	-0.01
5	1.01	0.01	65.80	1.82
7	1.01	0.01		
	2nd Amendment on day 10		2nd Amendment on day 6	
2nd Amend. (hours)				
0	1.03	0.01*[a]	1.03	0.01
2	1.19	0.08*	1.39	0.14
3	1.23	0.09*	1.21	0.08
4	1.27	0.10*	1.18	0.07
5	1.35	0.13*	1.19	0.07
6	1.44	0.16*	1.25	0.10
7	1.52	0.18*		
8	1.65	0.22*	1.58	0.20
10	1.87	0.27*		
12	2.14	0.33*	2.72	0.43*
14	2.52	0.40*	4.67	0.67*
16			38.46	1.59*
26	3.98	0.60	nd[b]	nd
30	4.39	0.64		

[a]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

[b]nd = not detectable

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$k = 0.063$, $R^2 = 0.99$	$k = 0.668$, $R^2 = 0.90$

DNS = data not sufficient to determine pseudo first-order rate constants

Time	Bacterial Concentration, cfu[a]/liter Replicate 1		Replicate 2	
1st Amend. (days)				
0	0.99×10^7		0.96×10^7	
2nd Amend. (hours)				
3	28.3*[b]		115.2	
7	24.3*	35.40 ± 15.89	781.1*	608.55 ± 244.02
14	53.6*			
16			436.0*	
31	14.6			

[a]cfu = colony forming units, average of three plates

[b]values with * used to determine second-order rate constants (K) in liters organism^{-1} hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$K = 1.79 \times 10^{-10}$	$K = 1.10 \times 10^{-10}$

DNS = data not sufficient to determine second-order rate constants

Figure C-7. Replicate Amendment Study, 1.0 ppm Quinoline, Walla Walla River - Madame Dorion Park (9-7-83)

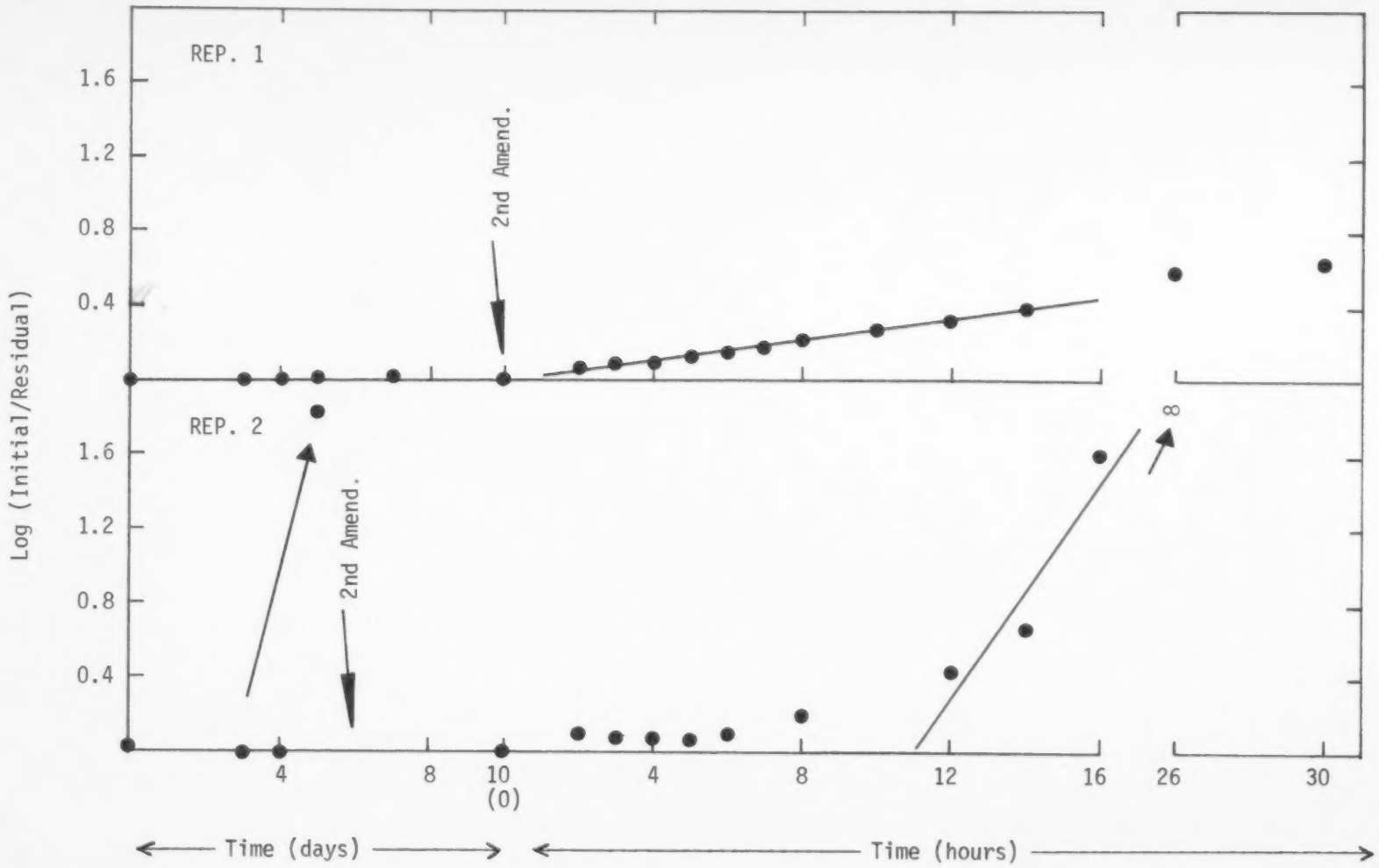


Table C-8. Replicate Amendment Study, 1.0 ppm Quinoline, Columbia River - 331 Building Fish Pond (9-7-83)

Incubation Time	Replicate 1		Replicate 2	
	Init./Res.	Log	Init./Res.	Log
1st Amend. (days)				
0	0.96	-0.02	1.01	0.003
3	1.18	0.07	1.33	0.12
4	1.01	0.005	1.08	0.03
5	1.03	0.01	1.04	0.02
		2nd Amendments on day 6		
2nd Amend. (hours)				
0	1.07	0.03*[b]	1.16	0.06
3	1.16	0.06*	1.47	0.17
4	1.14	0.06*	1.60	0.20*
5	1.19	0.08*	2.12	0.33*
6	1.56	0.19*	35.97	1.56*
7			nd	nd
8	1.51	0.18*		
11	2.04	0.31*		
12	2.52	0.40*		
14	2.42	0.38*		
16	nd[a]	nd		

[a]nd = not detectable

[b]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$k = 0.069$, $R^2 = 0.92$	$k = 1.57$, $R^2 = 0.82$

DNS = data not sufficient to determine pseudo first-order rate constants

Time	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
1st Amend. (days)				
0	1.49×10^7		2.59×10^7	
2nd Amend. (hours)				
3	24.6*[b]		4.3*	
7	33.1*	42.13 ± 23.40	88.9*	46.60 ± 59.82
16	68.7*		34.3	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$K = 1.64 \times 10^{-10}$	$K = 3.36 \times 10^{-9}$

Figure C-8. Replicate Amendment Study, 1.0 ppm Quinoline, Columbia River - 331 Building Fish Pond (9-7-83)

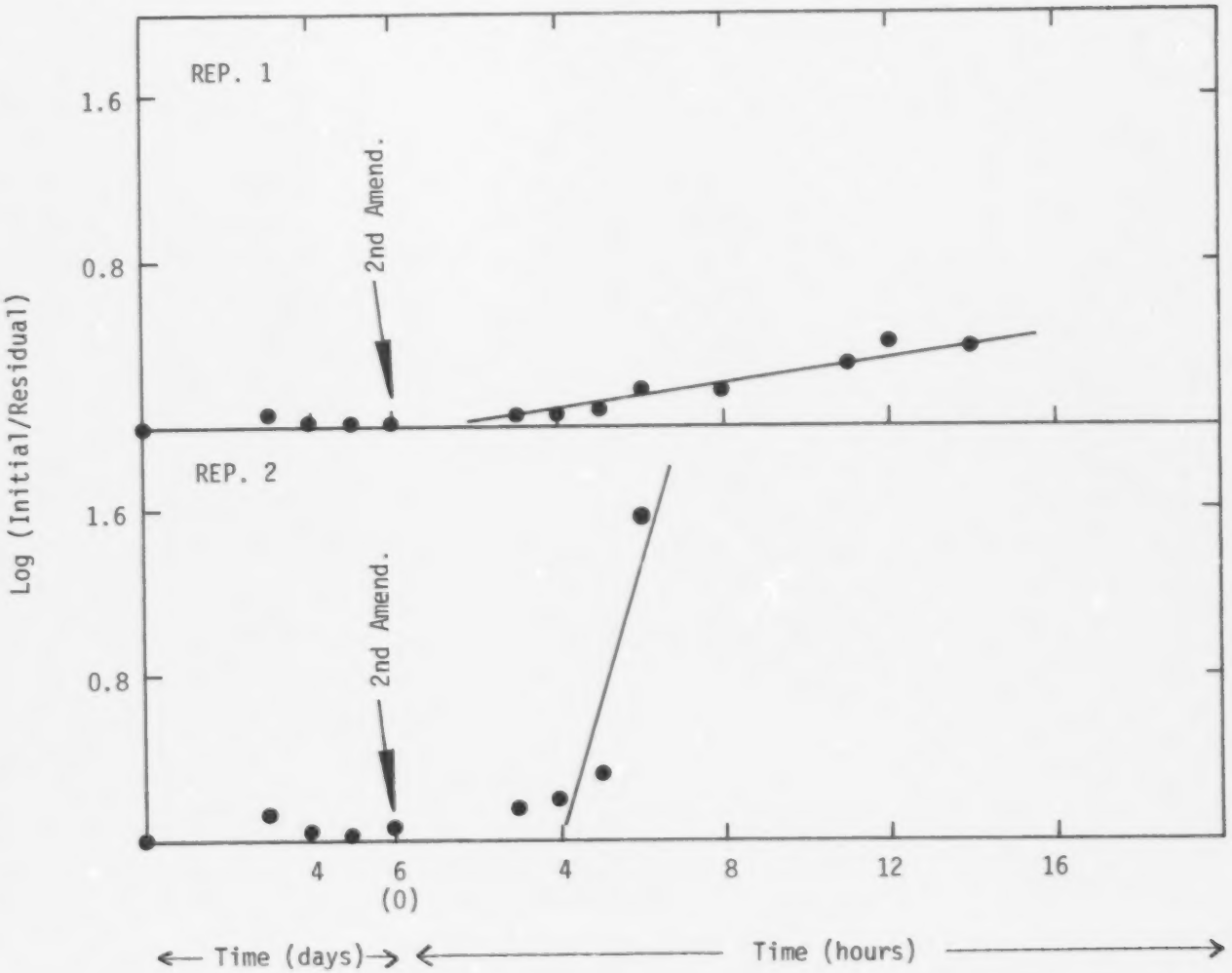


Table C-9. Replicate Amendment Study, 1.0 ppm Quinoline, Yakima River - Horn Rapids Park Boat Ramp (9-7-83)

Incubation Time	Replicate 1		Replicate 2	
	Tnit./Res.	Log	Tnit./Res.	Log
1st Amend. (days)				
0	0.96	-0.02	0.93	-0.03
3	0.91	-0.04	0.88	-0.05
4	1.96	0.29	1.51	0.18
5	nd[a]	nd	nd	nd
		2nd Amendment on day 10		
2nd Amend. (hours)				
0	0.88	-0.01	0.99	-0.01
2	0.99	-0.004	0.99	-0.01
3	1.00	0	1.00	0
4	0.99	-0.004	0.99	-0.002
5	1.00	0	1.01	0.01
6	1.01	0.004	1.03	0.01
7	1.03	0.01	1.04	0.02
8	1.04	0.02	1.06	0.03
10	1.08	0.03	1.08	0.03
12	1.11	0.05	1.09	0.04
14	1.15	0.06*[b]	1.11	0.05
26	1.41	0.15*	1.13	0.05
30	1.87	0.27*	1.15	0.06

[a] nd = not detectable

[b] values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$k = 0.027$, $R^2 = 0.87$	DNS

DNS = data not sufficient to determine pseudo first-order rate constants

Time	Bacterial Concentration, cfu[a]/liter			
	Replicate 1		Replicate 2	
1st Amend. (days)				
0	1.46×10^7		1.78×10^7	
2nd Amend. (hours)				
3	1.12		0.21	
7	1.83		2.33	
14	1.70*	1.97 ± 0.37	2.53	
31	2.23*		2.83	

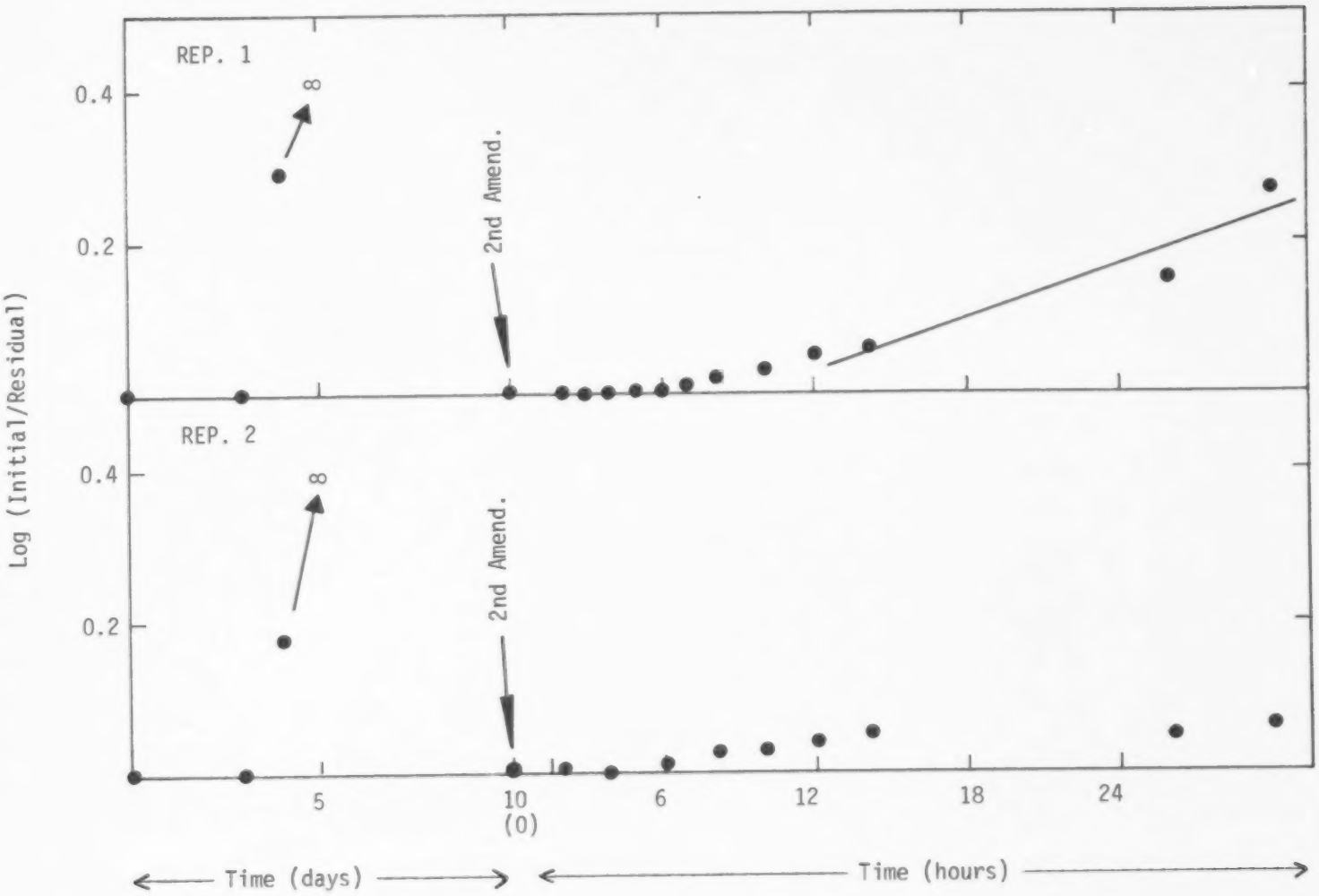
[a] cfu = colony forming units, average of three plates

[b] values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$K = 1.38 \times 10^{-9}$	DNS

DNS = data not sufficient to determine second-order rate constants

Figure C-9. Replicate Amendment Study, 1.0 ppm Quinoline, Yakima River - Horn Rapids Park Boat Ramp (9-7-83)



APPENDIX D

SECOND-ORDER RATE CONSTANTS FOR α-NAPHTHOL

Table D-1. Replicate Amendment Study, 1.0 ppm α-Naphthol, Yakima River - Horn Rapids Park Boat Ramp (5-2-83)

Incubation Time (days)		Replicate 1 Init./Res.	Replicate 1 Log		Replicate 2 Init./Res.	Replicate 2 Log
0		0.94	-0.03		0.96	-0.02
1		1.02	0.01		0.99	0.00
2		1.04	0.02		1.05	0.02
3		1.17	0.07		1.13	0.05
4		1.05	0.02		1.06	0.02
6		1.15	0.06*[b]		1.15	0.06*
7		1.95	0.29*		1.75	0.24*
8		1.27	0.10*		6.16	0.79*
9		4.19	0.62*		3.60	0.56*
10		nd[a]	nd		nd	nd
11		nd	nd		nd	nd
12	2nd Amend. →	0.73	-0.14	2nd Amend. →	0.67	-0.17
13		0.92	-0.04		0.92	-0.04
14		0.94	-0.03		0.75	-0.12
15		1.07	0.03*		1.20	0.08*
16		1.32	0.12*		1.08	0.03*
17		1.52	0.18*		1.18	0.07*
18		1.46	0.17*		1.07	0.03*
19		2.32	0.37*		1.80	0.25*
20		2.05	0.31*		1.94	0.29*
21		3.11	0.49*		2.26	0.35*
22		3.20	0.50*		2.33	0.37*
23		4.51	0.65*		3.52	0.55*
24		4.91	0.69*		3.28	0.52*
25		5.98	0.78*		4.23	0.63*
26		4.82	0.68*		5.77	0.76*
27		3.54	0.55*		3.09	0.49*
28		7.02	0.85*		6.86	0.84*

[a] nd = not detectable

[b] values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	$k = 1.43 \times 10^{-2}$, $R^2 = 0.57$	$k = 1.97 \times 10^{-2}$, $R^2 = 0.66$
2nd Amend.	$k = 5.65 \times 10^{-3}$, $R^2 = 0.88$	$k = 5.80 \times 10^{-3}$, $R^2 = 0.89$

Table D-1. (Continued)

Incubation Time (days)		Bacterial Concentration, cfu[a]/liter Replicate 1		Replicate 2	
1		6.53×10^6		6.83×10^6	
2		6.43		6.50	
3		12.33		5.47	
5		6.07		3.43	
6		2.03*[b]	6.12 ± 4.61	1.74*	5.28 ± 4.15
7		3.23*		1.93*	
8		6.90*		7.27*	
9		12.30*		10.17*	
12	2nd Amend. →	10.53	2nd Amend. →	29.57	
13		7.27		56.27	
14		17.07		38.20	
15		17.23*	9.51 ± 5.08	109.80*	121.09 ± 78.24
16		9.47*		102.00*	
19		10.13*		50.00*	
20		2.77*		322.00*	
21		8.97*		74.67*	
22		14.37*		106.67*	
23		5.87*		117.33*	
27		13.93*		97.67*	
28		2.87*		109.67*	
30		5.55		65.67	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters organism^{-1} hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	$K = 2.34 \times 10^{-9}$	$K = 3.73 \times 10^{-9}$
2nd Amend.	$K = 5.94 \times 10^{-10}$	$K = 4.79 \times 10^{-11}$

Figure D-1. Replicate Amendment Study, 1.0 ppm α-Naphthol, Yakima River - Horn Rapids Park Boat Ramp (5-2-83)

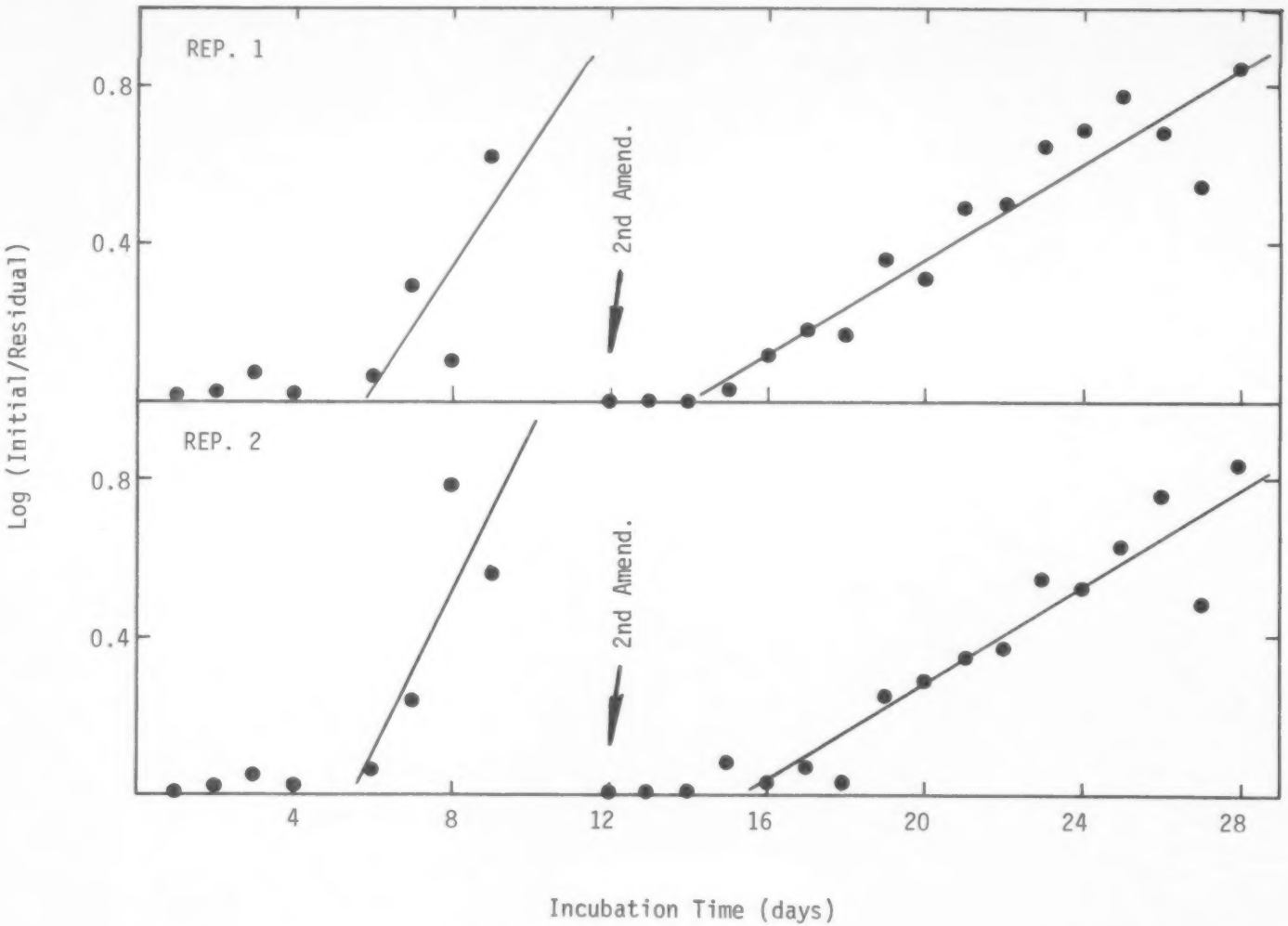


Table D-2. Replicate Amendment Study, 1.0 ppm α-Naphthol, Snake River - Hood Park Fish Pond (5-2-83)

Incubation Time (days)		Replicate 1 Init./Res.	Replicate 1 Log		Replicate 2 Init./Res.	Replicate 2 Log
0		0.94	-0.03		1.02	0.01*
1		1.05	0.02*[b]		1.14	0.06*
2		1.15	0.06*		1.26	0.10*
3		1.25	0.10*		1.30	0.11*
4		1.37	0.14*		1.44	0.16*
6		1.52	0.18*		1.53	0.18*
7		1.69	0.23*		1.82	0.26*
8		1.25	0.10*		2.95	0.47*
9		2.16	0.33*		2.54	0.40*
10		3.20	0.51*		3.49	0.54*
11		4.97	0.68*		3.07	0.49*
12		4.35	0.64*		6.11	0.79*
13		3.52	0.55*		4.07	0.61*
14		6.40	0.81*		14.09	1.15*
15		nd[a]	nd		nd	nd
16	2nd Amend. →	0.79	-0.10	2nd Amend. →	0.74	-0.13
17		0.80	-0.09		0.92	-0.04
18		0.87	-0.06		0.88	-0.05
19		0.90	-0.05		0.92	-0.04
20		1.22	0.09*		1.18	0.07*
21		1.26	0.10*		1.39	0.14*
22		1.32	0.13*		1.42	0.15*
23		1.73	0.24*		1.94	0.29*
24		1.82	0.26*		1.90	0.28*
25		1.98	0.30*		2.07	0.32*
26		2.72	0.44*		2.64	0.42*
27		1.67	0.22*		1.87	0.27*
28		2.90	0.46*		3.02	0.48*

[a] nd = not detectable

[b] values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	$k = 5.54 \times 10^{-3}$, $R^2 = 0.84$	$k = 6.17 \times 10^{-3}$, $R^2 = 0.84$
2nd Amend.	$k = 4.03 \times 10^{-3}$, $R^2 = 0.72$	$k = 4.16 \times 10^{-3}$, $R^2 = 0.80$

Table D-2. (Continued)

Incubation Time (days)		Bacterial Concentration, cfu[a]/liter Replicate 1			Replicate 2		
1		1.80*[b] x 10^6	1.75 ± 1.59		1.42* x 10^6	1.08 ± 0.79	
2		5.20*			3.23*		
3		1.43*			1.17*		
5		0.68*			0.71*		
6		1.79*			0.63*		
7		0.52*			0.33*		
8		1.44*			1.28*		
9		0.70*			0.88*		
12		4.40*			0.94*		
13		0.60*			0.82*		
14		0.72*			0.45*		
15		1.05			2.40		
16	2nd Amend. →	1.33		2nd Amend. →	1.92		
19		1.23			0.64		
20		18.37*	45.94 ± 20.55		11.40*	56.43 ± 34.45	
21		44.20*			41.53*		
22		43.47*			66.53*		
23		70.27*			63.47*		
27		68.67*			114.00*		
28		30.63*			41.67*		
29		140.67			70.00		

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters organism^{-1} hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	K = 3.17 x 10^{-9}	K = 5.71 x 10^{-9}
2nd Amend.	K = 8.77 x 10^{-11}	K = 7.37 x 10^{-11}

Figure D-2. Replicate Amendment Study, 1.0 ppm α-Naphthol, Snake River - Hood Park Fish Pond (5-2-83)

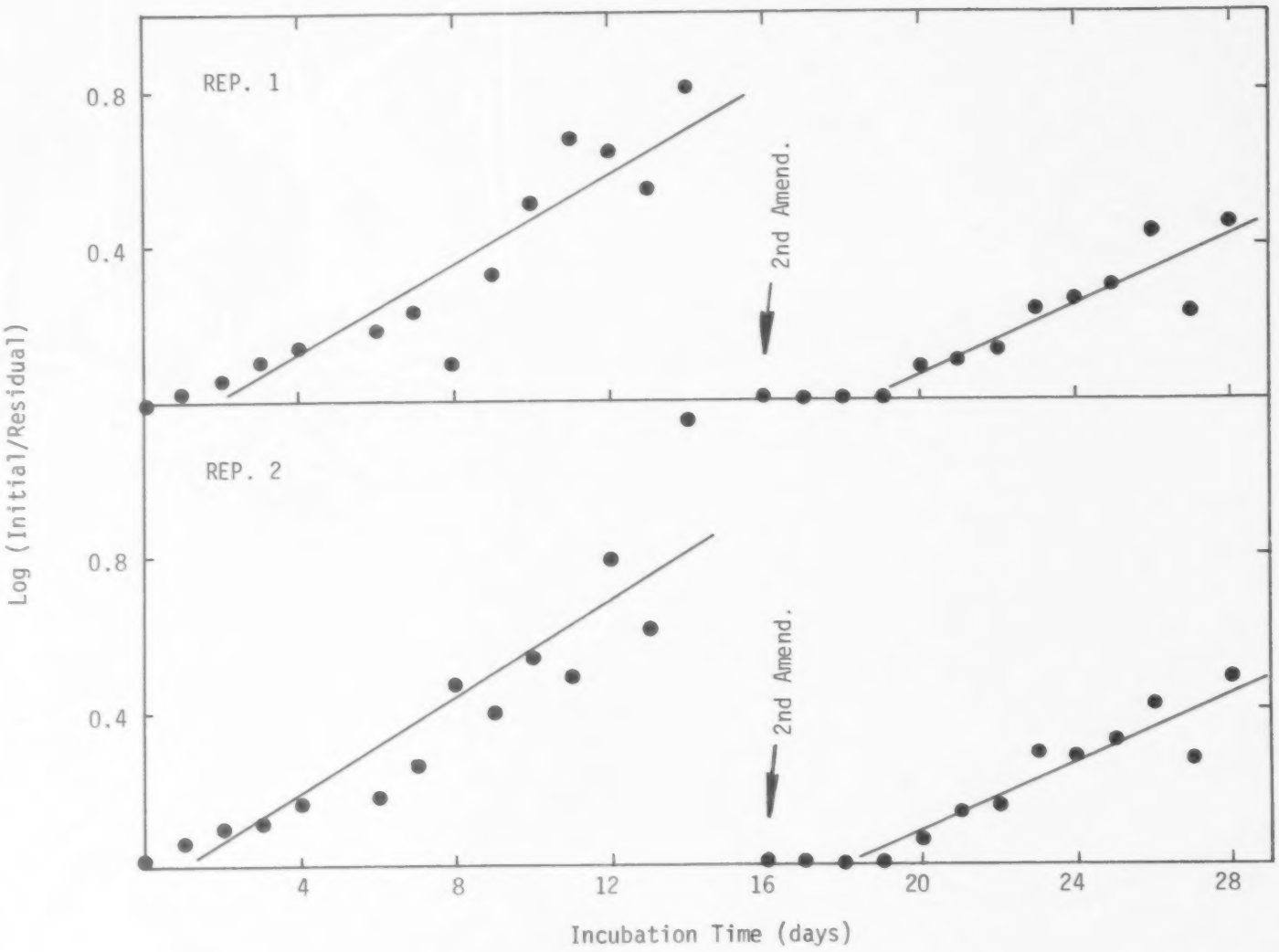


Table D-3. Replicate Amendment Study, 1.0 ppm α-Naphthol, Walla Walla River - Madame Dorion Park (5-2-83)

Incubation Time (days)		Replicate 1 Init./Res.	Replicate 1 Log		Replicate 2 Init./Res.	Replicate 2 Log
0		0.93	-0.03		0.94	-0.03
1		1.02	0.01		1.06	0.03
2		1.06	0.03		1.09	0.04
3		1.09	0.04		1.21	0.08
4		1.10	0.04		1.18	0.07
6		1.30	0.11		1.25	0.10*
7		1.19	0.08		1.26	0.10*
8		1.22	0.08* [b]		1.72	0.23*
9		1.61	0.21*		nd	nd
10		4.22	0.63*		nd	nd
11		nd [a]	nd		nd	nd
12	2nd Amend. →	0.63	-0.20	2nd Amend. →	0.70	-0.16
13		1.21	0.08*		1.58	0.20*
14		1.56	0.19*		1.73	0.24*
15		1.50	0.18*		5.36	0.73*
16		2.57	0.41*		nd	nd
17		2.04	0.31*		nd	nd
18		2.58	0.41*		nd	nd
19		3.13	0.50*	3rd Amend. →	0.70	-0.15
20		3.05	0.48*		0.66	-0.18
21		4.15	0.62*		1.02	0.01*
22		4.12	0.61*		1.15	0.06*
23		6.34	0.80*		2.18	0.34*
24		8.22	0.91*		2.61	0.42*
25		7.60	0.88*		5.03	0.70*
26		26.61	1.43*		5.10	0.71*
27		21.98	1.34*		6.07	0.78*
28		nd	nd		11.33	1.05*

[a] nd = not detectable

[b] values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	$k = 2.64 \times 10^{-2}$, $R^2 = 0.92$	$k = 6.24 \times 10^{-3}$, $R^2 = 0.75$
2nd Amend.	$k = 8.14 \times 10^{-3}$, $R^2 = 0.90$	$k = 2.54 \times 10^{-2}$, $R^2 = 0.81$
3rd Amend.	Third Amendment Not Done	$k = 1.40 \times 10^{-2}$, $R^2 = 0.96$

Table D-3. (Continued)

Incubation Time (days)	Bacterial Concentration, cfu[a]/liter Replicate 1		Replicate 2	
1	7.13×10^6		4.57×10^6	
2	5.57		14.50	
3	7.40		6.97	
5	3.10		2.77	
6	2.70		2.70*	7.47 ± 6.94
7	1.55		4.27*	
8	6.03*[b]	6.25 ± 0.31	15.43*	
9	6.47*		43.80	
12	2nd Amend. → 52.67		2nd Amend. → 38.53	
13	36.80*	12.17 ± 10.43	27.27*	20.26 ± 6.09
14	21.10*		17.13*	
15	15.00*		16.37*	
16	8.60*		6.77	
19	7.50*		3rd Amend. → 0.70	
20	2.10*		1.23	
21	3.33*		2.60*	49.33 ± 50.16
22	6.80*		18.87*	
23	6.10*		19.83*	
27	14.40*		116.67*	
28	1.47		88.67*	
30	19.97		76.67	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters organism^{-1} hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	$K = 4.22 \times 10^{-9}$	$K = 8.35 \times 10^{-10}$
2nd Amend.	$K = 6.69 \times 10^{-10}$	$K = 1.26 \times 10^{-9}$
3rd Amend.	3rd Amendment Not Done	$K = 2.84 \times 10^{-10}$

Figure D-3. Replicate Amendment Study, 1.0 ppm α-Naphthol, Walla Walla River - Madame Dorion Park (5-2-83)

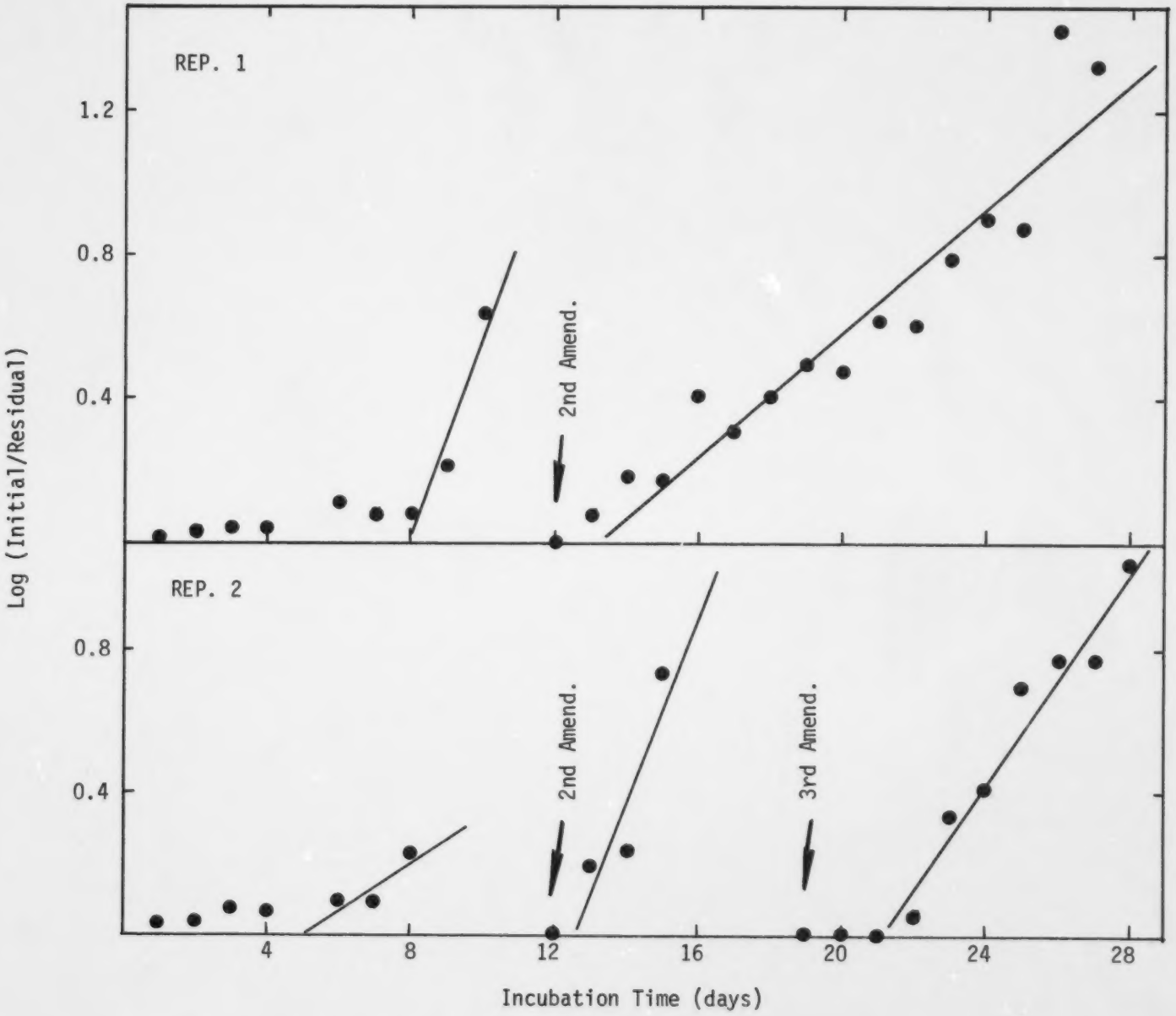


Table D-4. Replicate Amendment Study, 1.0 ppm α-Naphthol, Columbia River - Richland "Wye" Marina (8-19-83)

Incubation Time	Replicate 1 Init./Res.	Replicate 1 Log	Replicate 2 Init./Res.	Replicate 2 Log
1st Amend. (days)				
0	0.96	-0.02	0.96	-0.02
2	1.05	0.02	1.08	0.03
4	nd[a]	nd	nd	nd
7	nd	nd	nd	nd
		2nd Amendment on day 8		
2nd Amend. (hours)				
0	0.95	-0.02	0.94	-0.03
2	0.92	-0.03	0.97	-0.01
4	0.94	-0.03	0.95	-0.02
6	0.98	-0.01	0.98	-0.01
8	0.98	-0.01	0.96	-0.02
24	2.25	0.35*[b]	1.34	0.13*
28	5.65	0.75*	1.57	0.20*
32	48.50	1.69*	1.70	0.23*
48	nd	nd	2.00	0.30*
52			2.23	0.35*
56			2.37	0.37*
72			2.90	0.46*

[a]nd = not detectable

[b]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$k = 0.386$, $R^2 = 0.95$	$k = 0.015$, $R^2 = 0.98$

DNS = data not sufficient to determine pseudo first-order rate constants

Time	Bacterial Concentration, cfu[a]/liter Replicate 1		Replicate 2	
1st Amend. (days)				
0	1.22×10^7		0.59×10^7	
2nd Amend. (hours)				
4	4.07		6.97	
24	59.50*[b]	49.40 ± 14.28	21.90*	20.15 ± 2.47
54	39.30*		18.40*	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$K = 7.81 \times 10^{-10}$	$K = 7.34 \times 10^{-11}$

DNS = data not sufficient to determine second-order rate constants

Figure D-4. Replicate Amendment Study, 1.0 ppm α-Naphthol, Columbia River - Richland "Wye" Marina (8-19-83)

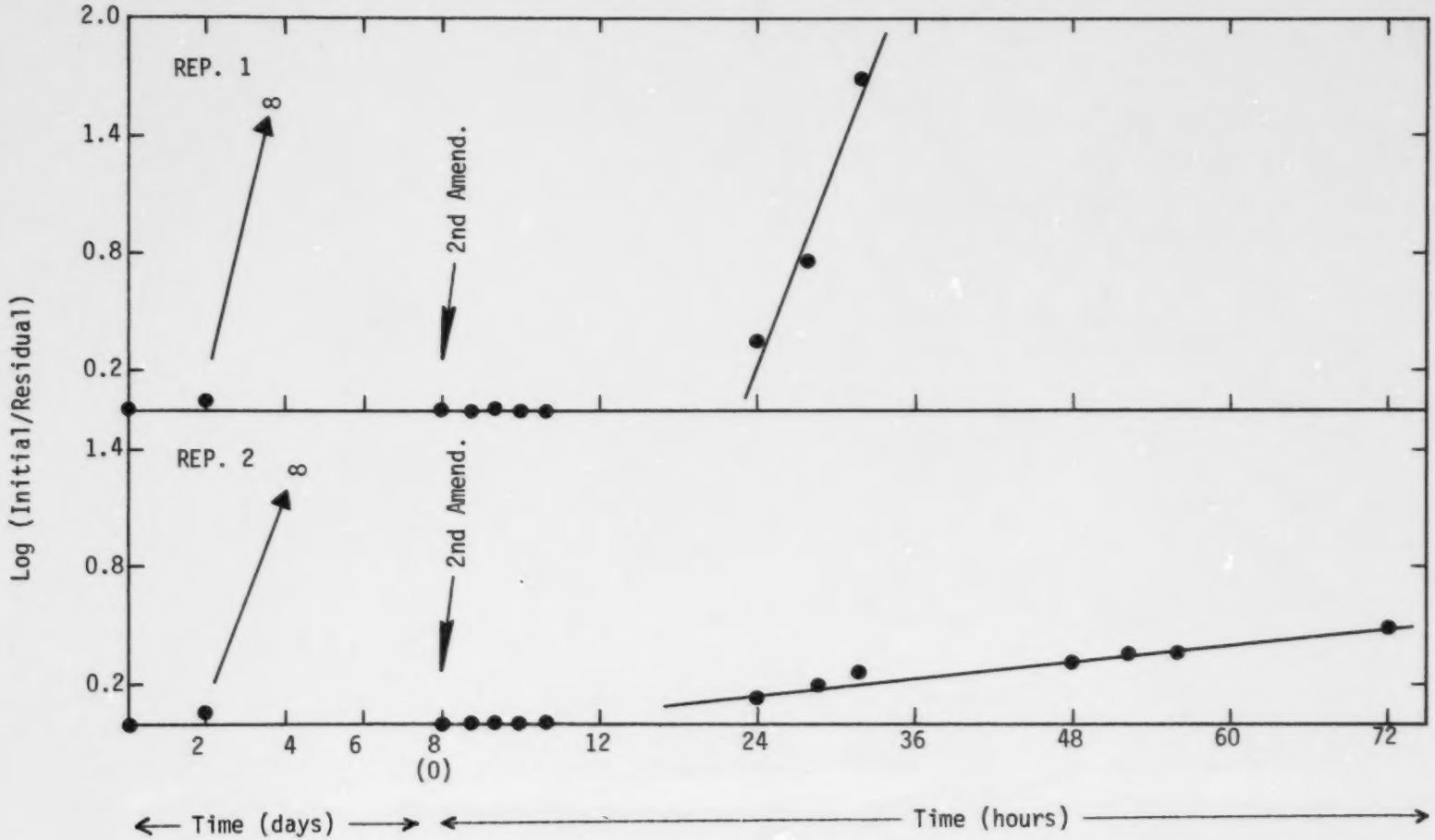


Table D-5. Replicate Amendment Study, 1.0 ppm α-Naphthol, Yakima River-Horn Rapids Park Boat Ramp (8-19-83)

Incubation Time	Replicate 1 Init./Res.	Replicate 1 Log	Replicate 2 Init./Res.	Replicate 2 Log
1st Amend. (days)				
0	1.04	0.02	1.04	0.02
2	1.28	0.11	1.28	0.11
4	1.45	0.16	1.69	0.23
7	nd[a]	nd	nd	nd
		2nd Amendment on day 8		
2nd Amend. (hours)				
0	0.87	-0.06	0.83	-0.08
2	0.86	-0.07	0.84	-0.07
4	0.88	-0.06	0.86	-0.07
6	0.95	-0.02	0.91	-0.04
8	0.93	-0.03	0.94	-0.03
24	3.35	0.52	nd	nd
28	nd	nd		

[a]nd = not detectable

Replicate 1	Replicate 2
DNS	DNS

DNS = data not sufficient to determine pseudo first-order rate constants

Time	Bacterial Concentration, cfu[a]/liter Replicate 1	Replicate 2
1st Amend. (days)		
0	0.34×10^7	0.48×10^7
2nd Amend. (hours)		
4	3.43	5.67
24	54.50	14.20
54	16.30	9.77

[a]cfu = colony forming units, average of three plates

Replicate 1	Replicate 2
DNS	DNS

DNS = data not sufficient to determine second-order rate constants

Figure D-5. Replicate Amendment Study, 1.0 ppm α-Naphthol, Yakima River - Horn Rapids Park Boat Ramp (8-19-83)

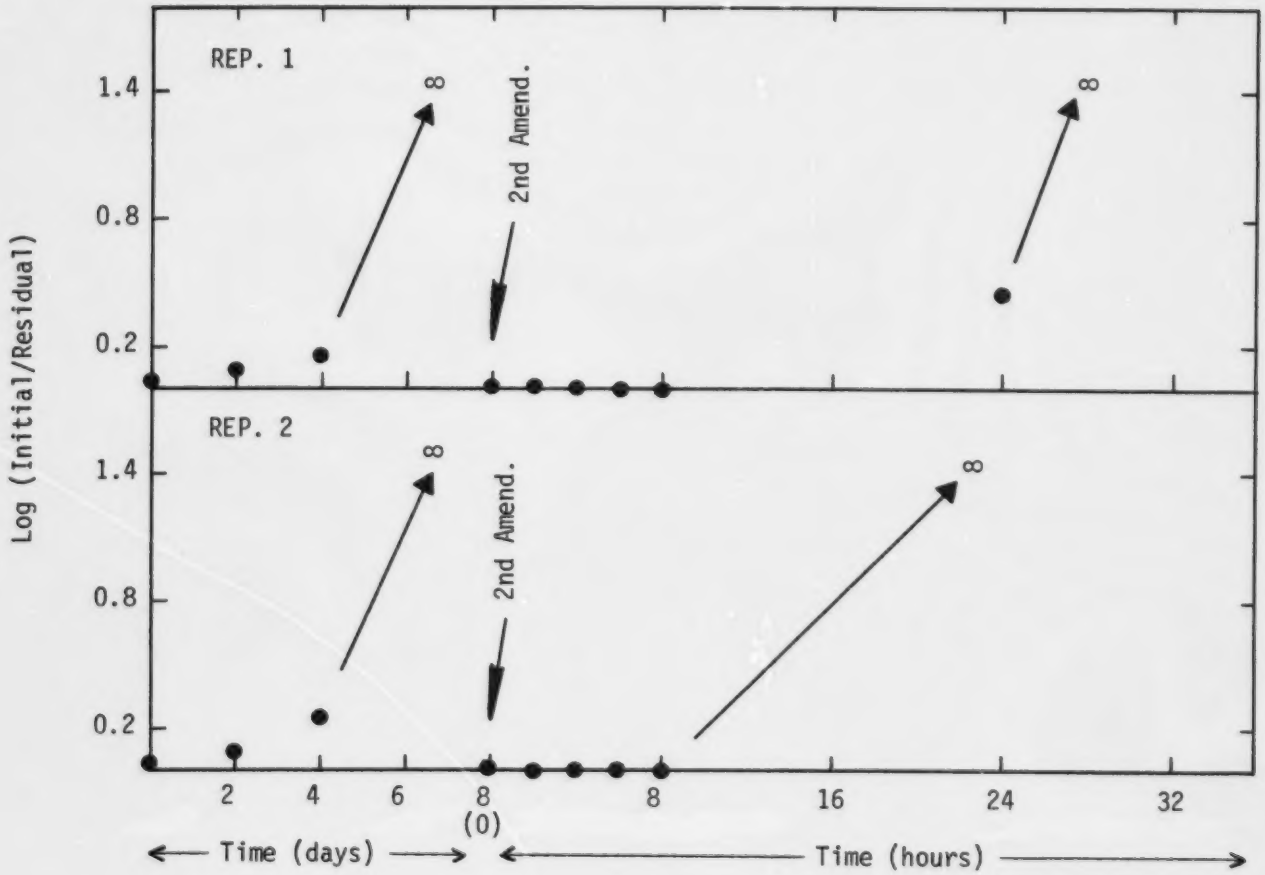


Table D-6. Replicate Amendment Study, 1.0 ppm α-Naphthol, 331 Bldg. Fish Pond (8-19-83)

Incubation Time	Replicate 1 Init./Res.	Replicate 1 Log	Replicate 2 Init./Res.	Replicate 2 Log
1st Amend. (days)				
0	0.99	-0.004	1.00	0.00
2	1.03	0.01	1.03	0.01
4	1.10	0.04	1.09	0.04
7	nd[a]	nd	nd	nd
		2nd Amendment on day 8		
2nd Amend. (hours)				
0	1.02	0.01	1.00	0.00
2	1.31	0.12	1.24	0.09
4	1.72	0.24*[b]	1.61	0.21*
6	2.83	0.45*	2.48	0.39*
8	7.85	0.89*	5.37	0.73*
24	nd	nd	nd	nd

[a]nd = not detectable

[b]values with * were used to determine pseudo first-order rate constants (k) in hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$k = 0.374$, $R^2 = 0.96$	$k = 0.299$, $R^2 = 0.97$

DNS = data not sufficient to determine pseudo first-order rate constants

Time	Bacterial Concentration, cfu[a]/liter Replicate 1		Replicate 2	
1st Amend. (days)				
0	4.40×10^7		2.33×10^7	
2nd Amend. (hours)				
4	99.20*[b]	159.60 ± 85.42	160.00*	190.00 ± 42.43
24	220.00*		220.00*	
54	219.00		172.00	

[a]cfu = colony forming units, average of three plates

[b]values with * were used to determine second-order rate constants (K) in liters $organism^{-1}$ hr^{-1}

	Replicate 1	Replicate 2
1st Amend.	DNS	DNS
2nd Amend.	$K = 2.34 \times 10^{-10}$	$K = 1.58 \times 10^{-10}$

DNS = data not sufficient to determine second-order rate constants

Figure D-6. Replicate Amendment Study, 1.0 ppm α-Naphthol, 331 Building Fish Pond (8-19-83)